JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXIV WASHINGTON, D. C., APRIL 21, 1923 No. 3

GUMMOSIS OF CITRUS[1]

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PART I.—GUMMOSIS DUE TO PYTHIACYSTIS CITROPHTHORA

INTRODUCTION

The purpose of Part I is to present the results of an investigation, begun in 1912, into the nature, causes, and manner of development of certain types of gummosis of Citrus trees. In the three parts of this paper the term gummosis will be employed in the broader sense, in which it applies not only to the process of gum formation but also to the diseases or pathological effects in which gum formation is one of the conspicuous features. When employed in connection with specific diseases, supplementary terms will serve to show its modified meaning.

Part I will deal mainly with the causal relation of *Pythiacystis citrophthora* Sm. and Sm. to one of the most widespread and destructive forms of Citrus gummosis in California. The relation of another fungus, *Phytophthora terrestria* Sherb., to a similar disease, mal di gomma, in Florida is also included. Part II will present the results of an investigation into the relation of *Botrytis cinerea* Lk. and other fungi to other types of gummosis in Citrus. The last section of this paper, Part III, will deal with gum formation as such, the conditions influencing its formation, and its relation to diseases. The results of investigations on the control of some of these diseases will be discussed in a bulletin to be issued from the California Agricultural Experiment Station and will therefore receive only a brief mention in this paper. A preliminary report regarding control has already been published by Fawcett, (*24,26*).

Previous investigators had come to the conclusion that all gum diseases of Citrus trees in California originated independently of microorganisms, according to Smith and Butler (*56*). It was held that these diseases were largely autogenous in their nature and were frequently induced through the effects of certain climatic or soil conditions alone. It now appears evident that these environmental conditions can not by themselves initiate all the severe forms of gummosis in Citrus earlier attributed to them, although many of these factors are found to play, as they do in most parasitic diseases, an important rôle as contributing

[1] Accepted for publication June 29, 1922. Paper No. 91, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] This investigation was started in the spring of 1912 under the auspices of the State commission of horticulture and was transferred to the University of California in the fall of 1913. The author wishes to acknowledge the encouragement given by Dr. A. J. Cook and Prof. R. E. Smith at the beginning of the work, the assistance rendered by J. D. Culbertson, J. A. Prizer, and many other Citrus growers. Acknowledgment is also due to Bruce Douglas for laboratory assistance during a portion of the time of the investigation and to those members of the staff of the College of Agriculture who aided by many suggestions and criticisms.

conditions which favor infection and invasion of the host by causal parasites.

Since, however, a nonparasitic explanation for these gum diseases in California had become so firmly established in the literature of the subject, it has been necessary to carry on a large series of carefully controlled experiments before concluding that fungi are necessary factors in the initiation and development of certain of these diseases. For the same reason the author deems it necessary to present his data in considerable detail.

The failure to recognize the parasitic factor in previous investigations, as regards Pythiacystis gummosis at least, is probably due in part to the following facts which will be brought out in more detail later: (1) The fungus initiating the disease is found in the bark in its vegetative stage only; (2) it dies out rapidly in tissue already invaded, leaving only a fringe or band of live mycelium at the advancing edges of the invaded tissues; (3) under adverse temperature and moisture conditions the organism frequently dies completely in the tissue, especially on resistant varieties, giving the appearance of a pure physiological effect; (4) in advanced stages the stimulus to gum formation spreads out far in advance of the band of invading mycelium, producing an outer gummous zone in which localized gum pockets are frequently formed. This outer gummous zone is usually free from the causal organism. Any attempt to find the organism in regions other than the narrow band at the outer rim of the invaded zone would result in failure.

HISTORICAL REVIEW OF CITRUS GUMMOSIS

In this historical review of the literature of Citrus gummosis it has been found impossible to separate with certainty the different forms of gummosis which recent investigations have shown to be distinct. It is believed, however, that most of the more destructive, rapidly developing forms mentioned by various writers were similar to those represented by Pythiacystis gummosis in California and mal di gomma in Florida.

Two of the earliest European writers on Citrus, Ferrari (*29, p. 156–158*) in 1646[3] and Sterbeek (*60, p. 177*) in 1682, briefly discuss certain forms of Citrus gummosis occurring in the orange plantings of Europe. Two other early writers on Citrus referred to by Savastano (*52*) as mentioning some minor form of gummosis were Clarici in 1726 and Corrado in 1787.

The first highly destructive type of gummosis of Citrus on record appeared in the Azores in 1834. Sweet-orange trees which had grown to the age of 200 to 300 years, and which were producing 6,000 to 20,000 oranges apiece, were found by Fouque (*32, p. 837*) to be affected with a very destructive form of gummosis (*1*). Yellow gum is mentioned as exuding on the trunks near and sometimes beneath the surface of the ground. The trees put on heavy crops of fruit, and the leaves turned yellow and fell off in great quantities. This brief description appears to indicate a form of disease resembling Pythiacystis gummosis. It was supposedly transferred from the Azores to the vicinity of Lisbon, Portugal, where a similar disease appeared in 1865.

A similar type of Citrus gummosis, according to Savastano (*52*), was extremely destructive in many Mediterranean localities. It was established in Messina, Italy, in 1863 and was reported near Reggio in 1864.

[3] Reference is made by number (italic) to "Literature cited," pp. 232–235.

The disease advanced rapidly from Messina, passing into the province of Catania to Acireale. In 1865 it was established at Palermo, raging severely there until 1870. In all the Sicilian Citrus orchards the gummosis was present in such intensity as to constitute a true epidemic which destroyed all the trees. The orchards were later replanted with sour-orange stocks. In 1870 the disease was established in the region of Genoa. It became scattered (not epidemic) in the orchards of the Naples, Amalfi, and Gargano regions. Briosi (*11*) estimates the damage from gummosis in Italy as $2,000,000 from 1862 to 1878.

In Greece, in Tunis, and in Spain gummosis became distributed in varying degrees of intensity. Briosi refers to its destructiveness in the Balearic Islands (near Spain) in 1871. In more recent years a similar disease has been reported in the Oasis of Tripoli by Leone (*44*) in 1918. Gummosis was attracting attention in Cape Colony, South Africa, in 1891.

In Australia a destructive gummosis is referred to by Alderton (*3*) as occurring in New South Wales between 1860 and 1870, by McAlpine (*46*) as occurring near Sydney in 1867 and in Queensland in 1876. It is reported by Kirk (*43*) in New Zealand in 1885.

In the United States, records place its appearance at about the year 1875 in California according to Mills (*48*) and 1876 in Florida by Curtiss (*17*). In Florida, as footrot or mal di gomma, it attracted serious attention in 1879, following a very wet year according to Hume (*41*). Moore (*49, p. 128–132*), in 1881, speaks of its recent appearance, and Swingle and Webber (*64*) in 1896 report it as still gradually spreading. In California, gummosis was a serious trouble in nearly every Citrus locality by 1878. It was spoken of by Garey (*34, p. 81–82*) as the only Citrus disease of importance at that time. The horticultural literature of this period indicates that the discontinuance of the use of the common lemon, lime, and citron as stocks and the general adoption of the sour orange and sweet orange as the principal stocks in California were due to this disease.

Some of the other localities in the American continents where gummosis has been an important disease are reported in Paraguay by Bertoni (*9*), in Brazil by Averna-Sacca (*5*), in Mexico by Gandara (*33*), in Cuba by Cook (*15*) and Cook and Horne (*16, p. 35*), and in Porto Rico by Stevenson (*62*).

Viewing the history of Citrus gummosis from the investigational standpoint, Briosi (*11*) studied a gummosis (mal di gomma) in Italy and described a fungus, *Fusarium limoni* Briosi, which he considered to be a factor in the development of the disease. McAlpine (*46*) regarded a similar type of severe gummosis in Australia as undoubtedly of an infectious nature and referred to the same fungus as the causal agent. His description of the disease indicates that it is similar in character to the form which Pythiacystis gummosis takes on large orange trees in California. It is quite possible that the "slender wandering filaments" which he found penetrating the tissue may have been those of a Pythiacystis-like fungus. In this connection it is of interest to note that in a letter to the writer in February, 1917, G. P. Darnell-Smith of the Department of Agriculture, New South Wales, reports finding *Pythiacystis citrophthora* on specimens of Citrus affected with a gum disease sent from the Norfolk Islands, east of Australia. Later, F. Stoward, in a letter of October 1917, reports having isolated *P. citrophthora* from lemon fruits in Western Australia and having confirmed its pathogenicity by inoculation.

Comes (*14*) produced gumming in Italy by inoculations with a bacterium which he called *Bacterium gummis* Comes. *B. gummis* is also mentioned by Averna-Sacca (*5*) in connection with gummosis of Citrus in Brazil and by Gandara (*33*) in connection with gummosis in Mexico.

A number of other investigators, among whom were Swingle and Webber (*64*), considered the severe gum diseases as probably infectious and due to some organism invading the bark, but little work of an experimental nature with Citrus appears to have been done until recent years. Fawcett (*22* and *23*) showed that a gumming of branches of Citrus in Florida was due to the presence of a fungus similar to *Diplodia natalensis* Evans. The same fungus was found by Burger (*28*) to be the causal agent in a twig disease of the peach.

Not alone in Citrus but in a number of other plants, especially Prunus, definite diseases accompanied by large gum exudations have been shown in recent years to be due to specific organisms. Among these may be mentioned forms of gummosis on cherries reported by Aderhold and Ruhland (*2*), Griffin (*37*), and Barss (*8*); on apricot and other deciduous fruits reported by Barrett (*6*); and on plum reported by Higgins (*39*).

There have been other investigators who concluded that the severe gum diseases in Citrus were due, not to the invasion of organisms, but to certain stimuli operating upon the affected parts. Savastano (*51*) made a comparative study of gummosis in both Prunus and Citrus and concluded, because the histology was the same in both genera, that gummosis in Citrus arose largely from wounds or traumatisms. This conclusion was in agreement with the views of many previous investigators as to gummosis in Prunus. Among these were Sorauer (*58*) in Germany, Prillieux (*50*) in France, and others. Savastano, in a number of papers in recent years on gummosis in Citrus, has modified this earlier view. He has distinguished clearly between mere gum formation as a general phenomenon and gummosis in connection with definite diseases. In one of these later publications (*52*) he accepts Comes's (*14*) conclusions as to the bacterial origin of the definite disease type and concludes that the aggravating conditions or causes influencing the occurrence of gummosis are lack of light, clayey, water-holding soils, level ground as compared to hillsides, excessive moisture about the roots, wounds from grafting or from digging about the roots and so forth. Most of these contributing conditions are those favorable for gummosis due to *Pythiacystis citrophthora* in California, or mal di gomma due to *Phytophthora terrestria* in Florida. In this connection it is of interest to note that R. E. Smith (*57*) found lemon fruits affected by typical brownrot like that due to *Pythiacystis citrophthora*, in a low-lying, poorly drained grove in Sicily, where footrot was very prevalent as mentioned by Fawcett (*25*).

Still other investigators have concluded that organisms are not at all involved in the initiation of gummosis but that certain conditions within or without the host are solely responsible for the diseases. Bertoni (*9*) in Paraguay appears to have considered Psorosis and a Pythiacystis-like form as phases of the same disease and concludes that poor condition of nourishment is the primary contributing cause. Later (*10*) he believed that shade was a corrective for gummosis. Grossenbacher (*38*) concluded that untimeliness of bark growth in connection with drought and low temperatures was related in some unknown way to gummosis of the mal di gomma type in Florida.

The previous views regarding Citrus gummosis in California, based in part on investigations on Pythiacystis gummosis, are fairly well indicated by the following quotation from Smith and Butler (*56*):

> The lesions, ulcers, or affected areas produced, are not primarily the seat of the trouble. They represent rather the effect of what may be called a general constitutional derangement showing itself by external outbreaks or symptoms at whatever points may chance to be most susceptible. ..... What may be called a primary weakness exists back of the visible symptoms, and in this weakness can be sought the fundamental cause and nature of the disease. It is, therefore, not necessary to identify a parasite, or strikingly evident climatic or soil conditions, to account for diseases of this class. ..... It may be said here without extended discussion, that in no case have we been able to recognize or demonstrate the presence of any fungus, bacterium, or other parasitic organism as the cause of any form of citrus gum disease.

Some of the paramount conditions mentioned by Smith and Butler as contributory to the occurrence of gum disease are the accumulation of soil against the trunk, excessive moisture where poor drainage or careless irrigation exists, the heaping of manure against the trunk, low budding, lack of loose aerated soil and sweet orange stock on heavy soils. It is now known that these conditions are favorable for infection by the fungus and the development of the disease.[4]

Previous investigations and observations on Citrus gum diseases had led, therefore, to three general hypotheses: (1) That gum diseases were brought about by organisms capable of infecting and invading the tissue under certain contributing conditions; (2) that gum diseases were due to wounds or other external stimuli other than microorganisms acting immediately on the parts affected; (3) that gum diseases arose autogenously being due to internal derangements of the host brought about by or without the influence of certain factors of the environment acting on the host as a whole.

PYTHIACYSTIS GUMMOSIS

SYMPTOMS

Pythiacystis gummosis, with its associated rot of the fruit, is probably the most widespread and destructive of the Citrus gum diseases. The most striking features of the disease on the common lemon,[5] which is the most susceptible form, are copious exudations of gum and large dead patches of bark on the trunk, followed by yellowing and dropping of leaves. On old sweet-orange trees and other partially resistant forms, the dead patches are usually smaller and show a greater tendency to become self-limited.

In the earlier stages of the disease (Pl. 1, A; 3, A; 5, A) the extent to which the bark is invaded by the fungus can only be determined by lightly scraping the thin surface of bark in the vicinity of the exuding gum until the green color is seen. The margin between the invaded tissue and the sound bark then shows only as a difference in color, the normal green shading off gradually into a drab. The bark is not softened but remains firm. After a considerable time it becomes sunken and begins to crack longitudinally.

[4] In this connection it should be mentioned that Smith (*55*) was also among the first to accept the conclusions to which the first experimental work by Fawcett (*24*) led, although these were opposed to the views which he had formerly held.

[5] The names of species and varieties of Citrus will be used in accordance with Swingle (*63*), as follows: common lemon, *Citrus limonia* Osbeck; rough lemon, a horticultural variety of *C. limonia* Osbeck; sweet orange, *C. sinensis* Osbeck; sour orange, *C. grandis* Osbeck; citron, *C. medica* Linn; trifoliate orange, *Poncirus trifoliata* Raf. The word lemon when used alone will refer to the common lemon and the word orange to the sweet orange.

On healthy, rapidly growing lemon trees the area of killed and darkened bark, elliptical to irregular in outline, is usually 15 to 30 cm. in vertical length and half that in width, when the gum first becomes apparent. By that time the fungus has been invading the tissue usually for a period of from two to four months. The removal of the bark at this time will show that the outer margin of the invaded zone is about coextensive with that seen on the surface (Pl. 3, B). Most often in young trees the death of the cambium and inner bark precedes slightly the death of the outer bark. The upward extension from the point of infection is usually many times its lateral and usually much greater than its downward extension.

In the cambium region surrounding an actively invaded area evidence of an influence extending from the margins of the dead bark will be found. There is simply a production of clear, watery gum which seems to originate in the region of the embryonic wood among the live cells without any apparent fermentation or decay. This region which is not yet darkened beyond the invaded portion, will be spoken of in this paper as the "outer gummous zone." It may in time extend considerable distances upward and downward and small distances laterally from the margin of the invaded zone. It has been traced for 60 and 90 cm. upward. The extent of this outer gummous zone varies especially with the age and rapidity of development of the disease lesion and the condition of the tree. In some cases it is much larger than the invaded zone and in others much smaller.

The inner surface of the bark in the invaded zone in a lesion of considerable size varies from mineral brown to burnt umber or fawn color [6] and the same discolorations will be found on the surface of the wood just at or beneath the cambium. (Pl. 3, A, B.) The discoloration does not extend far radially (usually only 2 to 5 mm.) into the woody layers. The cambium region in the outer gummous zone is chamois to yellow ochre in color, fading out gradually at the margins into the normal white color of the sound woody surface.

Frequently when the bark is irregular in contour, gum pockets will be formed, 2.5 to 5 cm. in longest axis, due to the rapid and unequal formation of gum. The gum accumulates near the cambium and by pressure separates the bark from the wood at certain places, forming definite pockets. The pressure is usually relieved by a break in the bark before the pockets become large. A few deeper gum pockets of considerable size have also been found, situated in the outer gummous zone, beneath layers of wood 3 to 6 mm. in thickness, showing accumulations of gum under pressure. The gum, which is watery and clear when first formed, hardens as it comes to the surface, apparently by loss of water, and finally becomes brittle. On the surface the hardened gum usually ranges from mahogany to chestnut in color.[6] The gum accumulates on the surface in long narrow ridges (Pl. 1, A, B; 4, A; 5, A, D; 7, A) or in oval masses, or runs down and collects in masses on the soil, depending upon the rapidity of formation and the dryness of the air. During periods of heavy dews and rains it gradually dissolves and disappears (Pl. 1, D; 5, E).

Only in rare cases where the surface of the bark is moist during the development of the disease is there any sign of fungus development to

[6] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

be detected without a microscope, and even then it is somewhat difficult to see because of the hyaline nature of the vegetative hyphae. The invading hyphae frequently die out rapidly behind the marginal fringe of advance, and quite often they die out completely over a part or all of this outer margin, so that progress of the disease is checked or entirely arrested. Such cases are often found in trees having some resistance, especially in orange and pomelo trees, or where the weather conditions subsequent to infection become unfavorable to the parasite.

In trees on which the disease has been present for a long time, the dead bark over the invaded portions dries, shrinks, and cracks. The larger cracks are mostly vertical, with smaller horizontal cracks (Pl. 1, D; 2, D, E; 5, E, F). A thin layer of the wood immediately under the invaded bark will usually be found to be infiltrated with hardened reddish brown gum which protects the under layers from rapid drying out and appears to protect the wood to a considerable extent against the entrance of wood-rotting fungi.

On old sweet-orange trees, the invaded areas are usually less extensive and more restricted laterally than on the common lemon. There is usually less gum than on the lemon. With the orange there is greater tendency than with the lemon for the invading fungus to die out—for the invaded area to become self-limited. Frequently the invaded areas on old sweet-orange trunks extend upward from the soil surface as narrow tongues of killed bark. On younger and frequently on older vigorous orange trees growing on heavy clay soils the disease may assume much the same characteristics as on the common lemon.

ISOLATION AND IDENTIFICATION OF PYTHIACYSTIS CITROPHTHORA

Culture tests were made from different parts of gummosis lesions, beginning at the center of a lesion and taking samples about 1 cm. apart upward, downward, and laterally from the center. A number of organisms were found, as would be expected, in the dead and discolored bark tissue. From recently killed and dying bark tissue at the advancing margin of the darker areas (invaded zone) a fungus was obtained which was afterwards found to be identical with that producing brownrot mentioned by Smith and others (57) of lemon fruits and which, even before its identity with *Pythiacystis citrophthora* was known, was shown to be capable of reproducing the same type of bark disease. Beyond the margin of the killed areas, however, all through the outer gummous zone the inner bark tissue was usually found to be entirely free from microorganisms, although internal gumming had set in 15 to 60 cm. above and somewhat less below the margin of the killed tissue.

Pythiacystis citrophthora was first isolated from the bark of diseased lemon trees in September, 1912, at Whittier, Calif., but its identity was not recognized at that time.

The method which the author finally found most useful for determining the fungus was to cut out with a sterile scalpel small bits of diseased tissue from the margin of the invaded portion and place them in tubes of corn meal agar. After two to four weeks' growth on this medium the characteristic sporangia were formed. By this method pure cultures were readily obtained, and when other organisms were present as contaminations *Pythiacystis citrophthora* could be identified by its characteristic sporangia. The Pythiacystis may readily be separated from contaminating fungi by transferring the mixed culture to a jar of water

containing lemon fruits. The Pythiacystis will enter the fruit and produce brownrot, leaving the associated organisms behind. It may then be isolated more easily as a pure culture from bits of the infected fruit.

After the discovery that *Pythiacystis citrophthora* was capable of inducing the disease it remained to be shown that this fungus was commonly present in connection with this type of gummosis. Accordingly, a survey was made of various Citrus localities in California. During the investigations (1912–1916) *P. citrophthora* was isolated 109 times from the diseased bark of 68 Citrus trees, representing about 30 different orchards in 10 counties of California, extending from San Diego on the south to Butte County on the north. It was also isolated from one locality in Arizona (Table I). In addition, the fungus was reisolated in 40 different cultures from 20 cases of gummosis produced by inoculation.

TABLE I.—*Isolation of Pythiacystis citrophthora from gummosis lesions from natural infections*

Variety.	Season.	Number of localities.	Number of trees.	Number of positive cultures.
Lemon	Spring	2	14	21
	Summer	8	19	32
	Fall	6	20	25
Sweet orange	Spring	5	5	12
	Summer	4	4	9
	Fall	2	4	4
Pomelo	Winter	1	1	4
	Spring	1	1	2

These 109 cultures were obtained from 53 lemon trees, 13 sweet-orange trees, and 2 pomelo trees. All of the lemon, 9 of the sweet-orange, and the 2 pomelo trees were affected with typical gummosis of the trunks with the invaded portions of bark extending some distance above the surface of the soil. Four of the orange trees had lesions low down, near or upon the main roots, as is typical of mal di gomma or footrot lesions.

Although many isolation culture tests were made from various portions of the affected bark, including the outer gummous zone, and even some of the unaffected tissue, all of the successful isolation cultures except one were obtained from the advancing margin or outer fringe of the invaded zone. Isolation tests were made every month in the year except during January and February, and cultures of the fungus were obtained during each of the 10 months. The secondary organisms most commonly obtained in cultural tests from the older portions of the invaded zone back of the margins were species of Fusarium, Alternaria, Cladosporium, and Colletotrichum, and a large number of bacterial species.

INOCULATIONS WITH DISEASED TISSUE

Even before the causal fungus was discovered the infectious nature of the disease was determined by means of a large number of inoculations with diseased bark, the results of which are represented by the following typical examples.

On February 27, 1912, at Chula Vista in San Diego County, the following inoculations were made on 16-year-old lemon trees in heavy soil. The inocula were inserted into vertical cuts about 2 cm. long, made

through the bark of healthy tree trunks after first washing the surface with water and with alcohol. Paraffined paper was then tied over the cuts, control cuts without inoculum being used for comparison. In these preliminary experiments, the inocula used were as follows: (1) Bits of diseased bark tissue from near the margin of an actively enlarging gummosis lesion; (2) a bit of gum-filled woody tissue taken just outside the area of killed bark, in the outer gummous zone; (3) a piece of exuded gum from the same lesion; (4) bits of bark from a sound, healthy tree (used as controls). A cut was also made without inserting inoculum of any kind.

Of these inoculations, the first (consisting of two trees) brought about a development of the disease beginning at the point of inoculation. The other cuts all healed without any exudation of gum or visible injury to the bark. It will be noticed that the inoculations resulting in disease were made from tissue cut from the advancing edges or margins of recently killed tissue, while the others were from tissue or gum beyond this killed area (outer gummous zone) or from healthy bark. The development of the disease in one tree will be described, since it proved to be typical of the severe form of Pythiacystis gummosis as it occurs on lemon trees in the coastal sections of California.

On April 24, 1912, about two months after inoculation, gum was exuding rapidly and flowing downward to the surface of the soil, 15 cm. below, and hardening in a 15-mm. ridge. On August 2, over five months after inoculation, the exterior area of discolored bark was 15 by 25 cm. with a copious exudation of gum, forming three ridges on the bark surface and with a large mass of gum on the surface of the soil (Pl. 1, A). The foliage on the inoculated side of the tree was beginning to turn yellow. On September 19, 1912, the area of killed bark had extended upward and laterally, until it occupied a space 30 by 30 cm. and covered one-third the circumference of the trunk (Pl. 1, B). On November 15, 1912, the bark was dead on half the circumference of the trunk and the gum had formed ridges on the surface. The leaves on the affected side of the tree were very yellow and were dropping. The foliage on the side not affected was still healthy and normal. This tree now presented the appearance typical of many trees in the same locality which were affected naturally with Pythiacystis gummosis. On March 11, 1913, a little over a year from the time of inoculation, only 10 cm. of the 90-cm. circumference of the tree contained live bark at the level of the greatest lateral extension of the disease. At the place of greatest extension upward (46 cm. from the surface of the soil), the disease was arrested and callus tissue had formed. On May 24, 1913, the invaded area had extended only slightly beyond that of the previous observation. Pieces cut from the margins of the drying bark (Pl. 1, D) yielded cultures of *Pythiacystis citrophthora*. Most of the gum first formed had been dissolved and carried away by the rains. The bark on the side first affected had dried and contracted, showing longitudinal fissures just like those seen in old naturally occurring cases. Gum was exuding on the margins of the killed bark. In every respect this was a typical case of the form of gum disease due to *P. citrophthora*. On September 3, 1913 (18 months after inoculation) only about 5 cm. of live bark remained on the circumference of the trunk (Pl. 1, C), and the tree was considered useless from a commercial standpoint.

Many other series of inoculations were made with tissue from Pythiacystis gummosis lesions in different localities, varying widely in climatic and soil conditions, with the same general results as to type and progress of the disease. Inoculated trees contracted the disease just as readily on light sandy soils in orchards in which Pythiacystis gummosis had never occurred before as in orchards on heavy soils in which the disease had previously prevailed (Pl. 1, E, F). Variations in the development of the disease in individual trees and at various seasons of the year are indicated in Table II, which summarizes some of the inoculations with tissue from diseased lesions.

TABLE II.—*Inoculations on trunks of 16-year-old lemon trees at Chula Vista, with tissue from diseased lesions*[1]

Experiment No.	Date of inoculation.	Date of observation.	Size of area.[2]	Gum formation.[3]
			Cm.	
		Mar. 24, 1912	—	3
		Aug. 2, 1912	25 by 15	3 (Pl. 1, A.)
		Sept. 19, 1912	30 by 30	3 (Pl. 1, B.)
1	Feb. 27, 1912	Nov. 15, 1912	33 by 30	1
		Mar. 11, 1913	48 by 80	—
		May 24, 1913	—	1 (Pl. 1, D.)
		Sept. 3, 1913	—	3 (Pl. 1, C.)
		Nov. 15, 1912	—	3 (Pl. 1, F.)
		Mar. 11, 1913	20 by 11	3
		May 24, 1913	38 by 11	3
2	Sept. 21, 1912	July 9, 1913	60 by 13	—
		Sept. 3, 1913	66 by 15	—
		Oct. 31, 1913	68 by 16.5	2
		Apr. 18, 1914	75 by 16.5	—
		Nov. 15, 1912	—	3 (Pl. 1, E.)
		May 24, 1913	49 by 22	2
		July 9, 1913	60 by 22	3
3	do	Sept. 3, 1913	66 by 22	—
		Oct. 31, 1913	70 by 22	—
		Apr. 19, 1914	84 by 22	—
		Feb. 6, 1913	—	3
		Mar. 11, 1913	13 by 8	—
		May 24, 1913	29 by 13	2
4	Nov. 16, 1912	July 9, 1913	46 by 33	3
		Feb. 6, 1913	—	0
		Mar. 11, 1913	7.5 by 5	1
		May 24, 1913	16.5 by 5	3
		July 9, 1913	16.5 by 5	—
5	do	Sept. 3, 1913	21.5 by 7.5	— (Pl. 7, A.)
		Oct. 31, 1913	23 by 10	3

[1] All experiments except No. 1 were made on trees growing in light sandy soil.
[2] The first number gives the greatest extension vertically and the second number the greatest extension horizontally. This applies also to similar data in the tables that follow.
[3] 0=none; 1=slight; 2=medium; 3=copious; a dash indicates that no data were obtained.

INOCULATIONS WITH DISEASED FRUITS

After it seemed probable that the fungus commonly associated with *Pythiacystis gummosis* was the same as that causing brownrot of lemon fruits, *Pythiacystis citrophthora*, it was of interest to determine whether the disease could be induced directly from the brownrotted fruits, either in wounds or on uninjured tissue.

Experiments were made (1) by inserting pieces of lemon fruits affected with the brownrot into incisions made in the bark of healthy lemon trees; (2) by placing similarly affected lemon fruits in contact with the roots or trunks of healthy lemon trees and covering them with moist soil; and (3) by placing affected lemons, as in experiment 2, at the root or trunk of orange trees budded on both the sour- and the sweet-orange stocks.

In a typical experiment of the first kind, a piece of diseased lemon rind was inserted into a cut in the bark of a 19-year-old lemon tree 20 cm. above the sweet-orange stock, on February 26, 1913. A control cut not inoculated healed rapidly without gumming, but in 45 days the inoculated cut showed definite evidence of infection. The killed bark area enlarged from 4 by 7.5 cm. in 70 days to 28 by 82 cm. in about 13 months after inoculation (Pl. 2, A, B, C). In about 18 months the killed area reached extreme dimensions of 35 by 125 cm., one-third of this length being upon the sweet-orange stock (Pl. 2, D); the leaves were now yellow and dropping on the affected side. Cultures were made from the bark and Pythiacystis was obtained, but only at the advancing discolored margins of the area where the bark was killed through to the wood. Specimens taken from other places in the diseased tissue, and from the live tissue just outside the diseased areas, either yielded other organisms or were sterile. Among the organisms found both inside and outside the area of recent invasion were species of Colletotrichum, Alternaria, and Cladosporium, and also species of bacteria.

The disease having been produced by inoculation from diseased fruits into cuts in the bark, it now became of interest to determine whether infection could originate from diseased fruit placed near uninjured bark.

On May 13, 1913, at Whittier, several lemon fruits affected with Pythiacystis-rot were buried in the soil near the trunk of a small lemon tree growing in a large earthen pot. The soil was heaped up against the trunk and kept moist by frequent watering. A similar tree in another pot received the same cultural treatment without the diseased fruit. During the summer of 1913 no results of infection were noted. When next examined, in March, 1914, the first tree was seen to be badly affected with typical Pythiacystis gummosis, while the control tree remained healthy.

In other tests on lemon and sweet-orange trees with decayed fruits, infection leading to Pythiacystis gummosis frequently but not invariably resulted. Sour-orange trees under the same conditions whether injured or uninjured failed to contract the disease.

These tests, taken in connection with numerous observations in orchards, show that the sour-orange tree is highly resistant to Pythiacystis gummosis and that sweet-orange and common-lemon trees, though easily infected through injuries, are not otherwise readily infected except under the most favorable conditions for invasion by the parasite.

INOCULATION WITH PURE CULTURES OF PYTHIACYSTIS CITROPHTHORA, AND REISOLATION

As a result of a large number of inoculations from pure cultures of *Pythiacystis citrophthora*, it was shown that this fungus was capable of invading the bark and bringing about all the characteristic effects that have been noted in naturally occurring cases or those produced by

inoculations with diseased bark or fruit. The following is a typical example.

On November 23, 1912, a lemon tree about 18 years old, having a trunk about 30 cm. in diameter, growing at Santa Paula, was inoculated by inserting a very small bit of mycelium from a culture of *Pythiacystis citrophthora* into a 2-cm. vertical cut made 15 cm. above the bud union. The surface of the bark had previously been washed with water and with alcohol and the inoculated cut was covered with oiled paper.

No effect from the inoculation was evident in 37 days, but in 42 days an invaded surface area of 5 by 9 cm. was observed, and this increased to 8 by 11 cm. in 76 days and to 8 by 35 cm. in 7 months, with copious exudation of gum (Pl. 3, A). A similar cut without inoculum, on the opposite side of the same tree, healed rapidly.

A strip of bark 10 by 68 cm., as seen in Plate 3, B, was cut out at this time, for the purpose of cultural examination and for enzym experiments, to be discussed in Part III of this paper. Cultures made from this strip of bark at various places, by cutting bits of tissue from the inner side, yielded *Pythiacystis citrophthora* at both the upper and the lower margin of the invaded zone (see white line shown on the plates) but not at points 25, 8, and 16 cm., respectively, above this killed area, within the outer gummous zone.

On removal of the bark, the cambium adjacent to the dark brown or blackened area appeared yellowish. This discoloration extended only slightly laterally but to a much greater distance from the margins of the cut upward and downward. In this outer gummous zone interior gum had formed in places 60 cm. or more upward from the killed and invaded margins. The causal organism, as indicated by isolation tests in numerous other cases, does not extend into this outer gummous zone. To remove the causal organism in treatment, therefore, it is necessary to cut away the bark only a short distance beyond the discolored region. Experiments have shown that when this is done the gum will cease forming, the further extension of the yellow, gummy zone will stop, and the bark over it will usually return to normal condition.

The cut-out area and surrounding bark shown in Plate 3, B, was painted with Bordeaux paste. Three weeks later, June 27, 1913, gum was seen to have continued to ooze out at the upper angle, but no further bark had died. The same tree is shown in Plate 3, C, in September, 1914, and in Plate 3, D, in June, 1920. No effect on the foliage or health of the tree, such as is usually seen in severe cases of Pythiacystis gummosis, was noticed in this tree. The organism was probably removed in time to obviate visible injurious effects.

The results of a number of other inoculations on lemon trees with cultures of *Pythiacystis citrophthora* are summarized in Table III; others on lemon, orange, and other Citrus varieties, will be found described under "Resistance" and "Mal di gomma." In all, about 90 inoculations with pure cultures resulted in Pythiacystis gummosis, from 20 of which the organism was reisolated.

TABLE III.—*Inoculations of Pythiacystis citrophthora on trunks of 19-year-old lemon trees at Santa Paula*

Experiment No.	Part of trunk inoculated.	Date of inoculation.	Date of observation.	Size of killed bark lesions.	Gum formation.[1]
1	30 cm. from bud union, 60 cm. from soil.	Nov. 23, 1912	Dec. 30, 1912	0	0
			Feb. 12, 1913	10 by 3.5	3
			Mar. 14, 1913	11.5 by 5	3
			Apr. 12, 1913	18 by 8	3
2	15 cm. above bud union.	.....do.....	Dec. 30, 1912	0	0
			Feb. 12, 1913	9 by 5	3
			Mar. 4, 1913	9 by 5	3
			Apr. 12, 1913	13 by 8	3
			May 7, 1913	13 by 8	3
			June 5, 1913	35 by 8	2 (Pl. 2, F, 3, A)
3	10 cm. above bud union.	.....do.....	Dec. 30, 1912	0	0
			Feb. 12, 1913	14 by 5	1
			Mar. 14, 1913	18 by 5	3
			Apr. 12, 1913	19 by 6.5	3
			May 7, 1913	19 by 6.5	–
			June 5, 1913	25 by 6.5	3
4	Trunk, 13 cm. above bud union.	Feb. 20, 1913	Apr. 1, 1913	.....	2
			May 7, 1913	7.5 by 4.0	3
			June 27, 1913	10 by 6	3
			July 28, 1913	15 by 8	3
			Oct. 23, 1913	33 by 15	3

[1] 0=none, 1=slight, 2=medium, 3=copious.

These typical examples show that the general development of the disease was the same with inoculations with pure cultures of *Pythiacystis citrophthora* as it was with the inoculation with bits of diseased bark or fruit previously described. A period of slow development for 2 to 4 months is followed by copious gumming and rapid development, resulting in large invaded areas in 6 to 10 months. Later the rate of increase is again comparatively slow and is frequently partially checked. It is believed that the accumulation of gum in the outer gummous zone is a material hindrance to the rapid advance of the fungus. This feature of the disease is discussed in Part III of this paper.

A partial representative list of inoculations from which *Pythiacystis citrophthora* was again isolated, with the date and the size of invaded areas at time of isolation, is shown in Table IV.

TABLE IV.—*Representative inoculations of Pythiacystis citrophthora*

Date of inoculation.	Date of reisolation.	Size of invaded area.
		Cm.
Nov. 16, 1912	Nov. 1, 1913	23 by 10
Do	July 10, 1913	46 by 15
Nov. 23, 1912	Apr. 12, 1913	47 by 8
Do	June 5, 1913	35 by 8
Do	.....do	25 by 6
Feb. 26, 1913	Feb. 7, 1915	95 by 46
Do	Oct. 22, 1913	33 by 13
July 13, 1913	May 9, 1914	48 by 15
Sept. 10, 1914	Oct. 9, 1914	15 by 5
Oct. 14, 1914	Apr. 7, 1915	25 by 8
Feb. 7, 1915	Sept. 3, 1915	8 by 5
Mar. 4, 1915	.....do	30 by 10
Do	.....do	46 by 13
Apr. 9, 1915	.....do	30 by

As is shown by Table IV, the fungus was recovered in different cases from 1 to nearly 12 months after inoculation and from margins of different invaded areas varying from 8 to 95 cm. in vertical extent. Since the fungus was isolated most frequently at the upper margin of the invaded area, the distances from the original point of inoculation at which the fungus was recovered was frequently more than one-half that of this vertical extension, because the areas usually enlarged upward much faster than downward or laterally, as previously pointed out.

The severe inoculation test to which this fungus was subjected in order to establish its causal relation to this type of gummosis is indicated by an example of the repeated isolations, inoculations and reisolations of one of the strains of this fungus, over a period of three years. Isolated in September, 1912, its record was as follows:

Date of inoculation.	Date of reisolation.	Size of lesions.
		Cm.
Nov. 23, 1912	Apr. 12, 1913	47 by 8
June 13, 1913	May 9, 1914	48 by 15
Mar. 4, 1915	Sept. 3, 1915	46 by 13

The fungus was thus in the bark of the three trees for periods of about 5, 11, and 6 months, respectively, and between these periods in cultures for about 2, 2, and 10 months, respectively. Transfers from the original culture which were kept alive for over 8 years, on cornmeal-agar medium, were still capable of producing brownrot of lemon fruits when tested in 1921.

INOCULATIONS WITH PYTHIACYSTIS CITROPHTHORA ON DIFFERENT PARTS OF SAME TREES

After the results of the first inoculations began to indicate that *Pythiacystis citrophthora* was the causal agent in this type of disease, it became important to find out the effect of the fungus when introduced into different parts of the same tree. Inoculations were made on roots, trunk, and branches of various ages, as shown in Table V.

TABLE V.—*Inoculations with Pythiacystis citrophthora July 13, 1913, at different locations on same tree*

Part of tree inoculated.	Length and width of killed bark areas.					Final extreme distance from place inoculated.	
	1913			1914			
	July 27.	Sept. 13.	Nov. 25.	Apr. 9.	May 9.	Upward.	Downward.
Large root (sweet-orange stock), 13 cm. below bud union (Pl. 4, B)	*Cm.* 2.5 by 1.8	*Cm.*	*Cm.*	*Cm.* 13 by 4	*Cm.* 14 by 5	*Cm.* 9	*Cm.* 5
Orange bark of stock 5 cm. below bud union (Pl. 3, D)	4 by 1.8	10 by 2.5	24 by 10	43 by 13	48 by 15	40	8
Lemon trunk, 13 cm. above bud union (Pl. 4, B)	5 by 2.5	25 by 10	50 by 25	53 by 25	68 by 25	46	22
Lemon trunk 88 cm. above bud union just below crotch	2.5 by 0.6	13 by 4	34 by 9	36 by 9	36 by 9	.....	.....
Limb 8 cm. in diameter	2.5 by 0.6	20 by 6	33 by 8	33 by 8	33 by 8	20	13
Limb 5 cm. in diameter	.....	.....	30 by 5	30 by 5	30 by 5	18	12
Limb 5 cm. in diameter	.....	.....	27 by 1.3	30 by 2.5	30 by 2.5	20	10
Limb 2.5 cm. in diameter	.....	10 by 5	10 by 5	15 by 5	15 by 5	10	5
Limb 3 mm. in diameter	.....	15 by 5	18 by 5	22 by 5	22 by 5	15	7
Controls, not inoculated	0	0	0	0	0	0	0

The control cuts not inoculated healed without gumming or dying of bark. All cuts inoculated with *Pythiacystis citrophthora* resulted in killing of the bark accompanied by exudation of gum. The areas on the branches had nearly reached their maximum size when examined on November 25, 1913, 104 days after inoculation. A slight increase in some of them took place previous to the next April, at which time they had ceased to enlarge and had become self-limited, as was shown by subsequent records in Table V. The fungus apparently died out of these limb lesions, as no evidence of it could be found later.

The inoculation on the orange root resulted in gumming and killing of some bark in two weeks and continued gumming for about four months. This lesion (Pl. 4, C, lower area) became self-limited after an area 14 by 5 cm. had been killed and later resembled self-limited typical footrot or mal di gomma lesions as they often occur naturally on orange trees in Florida.

The diseased area resulting from inoculation on the orange bark 5 cm. below the bud union enlarged at about the same rate as that on the root for the first two weeks. After it had spread to the lemon bark, however, at the bud union, the progress upward was rapid. By May 9, 1914, the killed area was 48 by 15 cm. (Pl. 4, A, B), only 8 cm. of this extension being downward on the orange bark. Of five cultures taken about 10 months after inoculation from the margin of the invaded area (see chalk line on Pl. 4, A), three developed *Pythiacystis citrophthora*. Further extension of the area was prevented by cutting out the bark (Pl. 4, B) and painting the trunk with Bordeaux paste.

The result of the inoculation made on the lemon bark on the opposite side of the same tree a few centimeters above the bud union is shown in Plate 4, C, the killed area being at this time (April 9, 1914) 53 by 25 cm. with large quantities of gum exuding on the surface.

COMBINED EFFECTS OF PYTHIACYSTIS CITROPHTHORA AND FUSARIUM SP.

During the examination of a large number of naturally occurring cases of Pythiacystis gummosis it was noticed that a species of Fusarium frequently accompanied and was closely associated with *Pythiacystis citrophthora* in the diseased tissue. The question arose as to whether the Fusarium played any part in the development or the severity of the disease.

Fusarium has been mentioned frequently in literature as having some possible relation to certain types of gum disease. Briosi (*11*) and McAlpine (*46*) concluded that *Fusarium limoni* Briosi played an important part in mal di gomma in Italy and Australia. Earle and Rogers (*21*), though not able to produce gummosis by inoculation with Fusarium, believed that under certain conditions it was probably a factor in a certain type of gum disease in Cuba. The writer had previously also found species of Fusarium repeatedly associated with mal di gomma or footrot in Florida, but inoculations with them had been negative.

Cuts, as before, into which bits of pure cultures of *Pythiacystis citrophthora* and *Fusarium* sp. were placed side by side were now made on lemon trees. At the same time inoculations into other similar trees were made with *P. citrophthora* alone and others with *Fusarium* sp. alone. The main results are given in Tables VI and VII.

TABLE VI.—*Inoculation tests with Pythiacystis citrophthora alone, compared with those with P. citrophthora plus Fusarium sp.*

INOCULATIONS MADE LOW ON TRUNKS OF 19-YEAR-OLD LEMON TREES, FEBRUARY 20, 1913

Date of observation.	*P. citrophthora* alone.		*P. citrophthora* and *Fusarium* sp.	
	Size of lesions.	Gum formation.[1]	Size of lesions.	Gum formation.[1]
1913.	*Cm.*		*Cm.*	
Apr. 1	0	1	0	1
May 7	7.5 by 3.8	3	6.3 by 2.5	3
June 27	10 by 6.3	3	13 by 4	3
July 28	15 by 7.5	3	30 by 9	3
Oct. 23	33 by 13 (treated).	3	No record	
Nov. 25			58 by 18	3

INOCULATIONS MADE HIGH ON TRUNKS OF 21-YEAR-OLD LEMON TREES, FEBRUARY 25, 1915

1915.	*Cm.*		*Cm.*	
Mar. 4	0	0	0	0
Apr. 21	2.5 by 2.5	1	5 by 2.5	3
May 18	6.5 by 2.5	2	9 by 7	3
Sept. 4	8 by 5	2	38 by 15	3

[1] 0=none; 1=slight; 2=medium; 3=copious.

TABLE VII.—*Inoculation with Fusarium sp. at Santa Paula.*

Experiment No.	Date of inoculation.	Maximum results as to—		
		Gum.[1]	Initially killed bark at inoculation point.[1]	Cracking of outer bark.[1]
1	Nov. 23, 1912	2	1	1
2	.....do	1	0	1
3	Feb. 20, 1913	0	1	0
4	.....do	0	1	2
5	Mar. 14, 1913	1	1	1
6	.....do	0	1	1
7	.....do	1	1	1
8	Feb. 25, 1915	0	1	0
Control cuts without inoculum, to correspond with each of the above		0	0	0

[1] 0=none; 1=slight; 2=medium.

The progress of the disease on the tree inoculated with Pythiacystis and Fusarium on February 20, 1913, is more completely shown by Plate 5. The invaded area which was 58 by 18 cm. on November 25, 1913 (Pl. 5, B), the last date given in Table VI, had increased to 90 by 41 cm. on February 6, 1915 (Pl. 5, C–F). Extension vertically remained at 90 cm., but the lateral extension had increased to 61 cm. by March 9, 1916; and subsequently the entire circumference was encircled and the tree killed by the disease.

In the comparative inoculations with Fusarium alone on February 20, 1913, and February 25, 1915, No. 3 and 8 (Table VII), only a narrow layer of tissue along the cuts was killed, without exudation of gum. A thin outer layer of bark subsequently died about one inoculation, but otherwise the effect was not different in either case from that produced by the uninoculated cuts on the same trees. In a number of other inoculations with species of Fusarium associated with gummosis shown in Table VII, only slight effects in killed tissue and only slight to medium effects in gum formation were obtained.

Although the experiments on this phase of the question have been too few as yet to justify definite conclusions, the results suggest that the severity of the disease may be slightly increased by adding *Fusarium* sp. to *Pythiacystis citrophthora* at the time of inoculation. The characteristics of the disease except in rapidity of development, however, were the same as when *P. citrophthora* was inserted alone.

RESISTANCE OF DIFFERENT SPECIES AND VARIETIES OF CITRUS TO PYTHIACYSTIS GUMMOSIS

Among the Citrus species and varieties that have been tested, the common lemon has the lowest resistance to Pythiacystis gummosis and the sour orange the highest. The sour orange usually is so resistant to Pythiacystis attack that even when the most favorable conditions are given by inoculation in wounds there is only a slight gumming with rapid healing of the wounded tissue and with total failure to produce a diseased lesion. The sour orange is also highly resistant to all other infectious gum diseases of importance. Mere gum formation, however, may be induced by suitable stimuli in sour orange as well as in other species and varieties. Of the forms which have been most used for stocks, the trifoliate orange probably stands next to the sour orange in resistance and the sweet orange next to the common lemon in susceptibility, with the pomelo and the rough lemon between these two. Because these stocks are grown from seed there is a large possibility of variation in resistance within each variety, due to differences between "strains," and observations have indicated that such variation actually exists. The following observations and experiments indicate in a rough way the relative resistance of some of the species and varieties.

A block of 5,000 sweet-orange seedlings about 2½ years old, growing in nursery rows on medium heavy clay loam soil, had been planted adjacent to a block of 15,000 sour-orange seedlings of the same age and having the same care. All the trees had been irrigated rather frequently and heavily. On October 21, 1914, four representative rows of sweet-orange trees showed the following percentage of *Pythiacystis gummosis:*

Row No.	Number of trees in row.	Number of affected trees.	Percentage affected.
1	222	52	23
2	213	73	34
3	212	63	29
4	180	53	29
Total	827	241	.....
Average	.....	.....	29

On some trees only a small lesion was evident with much gum exuding, on others the bark was killed to a distance of 15 to 30 cm. above the soil, with an abundance of gum, and still other trees were dead. Some trees showed a strong tendency to form ridges of callous tissue along the edges of the dead strips of bark. A thorough search in the block of sour-orange trees failed to reveal a single tree affected.

Differences in resistance are indicated further by an estimate made by W. M. Mertz in a nursery of Citrus seedlings about 2 years old grown at the Citrus Experiment Station. The following is the percentage of gum disease (probably *Pythiacystis gummosis*) which was recorded:

Species.	Number of trees.	Percentage with gummosis.
Citrus aurantium (sour orange)	1,000	0.3
Poncirus trifoliata (trifoliate orange)	1,000	1.0
Citrus grandis (pomelo)	1,000	2.5
Citrus sinensis (sweet orange)	2,000	10.0

The inoculations recorded in Table VIII were made on trees about 2 years old from seed, by cutting through the bark on the stem not far from the soil and inserting bits of the mycelium from cultures of *Pythiacystis citrophthora* grown on sterilized orange wood. Oiled paper was tied over the cuts.

TABLE VIII.—*Inoculations made May 15, 1916; observations July 2, 1915, when the plants were pulled up*

Host.	Number of plants.	Results.
Sour orange	3	No gum on exterior. One shows interior gum. All healing normally.
Rough lemon	5	No gum. Healing normally.
Sweet orange	4	Two trees gumming copiously; long strip of bark killed. Two trees no gum on surface; wounds healing.
Pomelo	3	Bark killed slightly on edges of cuts without gum on exterior; healing rapidly.

Of the four forms tested in this experiment, sweet orange showed the greatest effect, pomelo less, and sour orange and rough lemon[7] showed no appreciable effect from the inoculation. The weather was very hot and dry during the experiment and thus not very favorable to the fungus.

On February 6, 1915, inoculations into the trunks of 21-month-old seedling sour-orange and rough-lemon trees were made at Santa Paula. A branch of a young common-lemon tree close by was inoculated at the same time. The results on May 18, 1915, were as follows:

The inoculated sour-orange cuts healed rapidly, with slightly more gaping of the wound than in the controls, while the rough lemons showed a small amount of dead bark next to the inoculated cuts, which were also healing rapidly. A slightly lower resistance of the rough lemon than of the sour orange was indicated. On the common lemon, however, the leaves had withered on the inoculated branch, the bark was killed

[7] The rough lemon is thought to be a hybrid. It is used largely for stocks in Florida and should not be confused with the common lemon.

around the branch for a distance of 8 cm. below the wound, and a large quantity of gum had formed. The control cuts on all three varieties healed perfectly without gumming or evident injury to the trees.

On July 2, 1913, inoculations were made at Whittier, with *Pythiacystis citrophthora* in cuts on the bark of sour-orange, sweet-orange, and a number of young deciduous fruit trees. The results on September 26, 1913, are given in Table IX.

TABLE IX.—*Comparative inoculations with Pythiacystis citrophthora into Citrus and deciduous fruit trees. Inoculations made July 2, 1913; observations, September 26, 1913*

Inoculation No.	Host.	Results.
1	Sour-orange stock (Valencia orange scion above).	Slight gumming, but healing rapidly.
2	Sour-orange stock (Valencia above)...	Healed perfectly, no gumming.
3	Sour-orange stock (lemon above).....	Healed with swollen scar only.
4	Valencia orange scion, hardened tissue (sour-orange stock below).	Gumming copiously with killing of small amount of tissue.
5	Valencia orange, same tree as No. 4, younger tissue.	Gumming copiously; 15-cm. strip of bark on one side of twig killed.
6	Control cuts on sour-orange and on Valencia orange bark (not inoculated).	All healed perfectly without visible gumming on surface.
7	Almond (2 trees).....	Gum exuded. Tissue slightly killed beyond cut.
8	Almond (control, not inoculated).....	Healed perfectly without gumming.
9	Peach (12-mm. stem).....	Much exuded gum. Bark killed 5 by 1.3 cm.; killed wood 8 cm. long.
10	Peach (control, not inoculated).....	Healed perfectly with slight gumming
11	Burbank plum.....	Gumming, outer part of bark killed and sunken, but healing underneath.
12	Burbank plum (control not inoculated).	Healed perfectly, no gum.
13	Pear.....	Healed with enlarged scar.
14	Pear (control).....	Healed with slight scar.

As before, the sour-orange bark showed high resistance while the sweet-orange (Valencia) bark was considerably affected by the fungus. Of the deciduous fruits tested, peach was most affected, almond and plum slightly, and pear scarcely at all.

In Table X are shown the results of inoculation into branches of various species by placing bits of Pythiacystis-infected fruit into cuts.

TABLE X.—*Comparative inoculations on Citrus branches, October 9, 1914; observations October 22, 1914*

Experiment No.	Host.	Size of diseased lesions.	Gum.[1]
		Cm.	
1	Common lemon.....	5 by 1.3	3
2	.....do.....	5 by 1.3	3
3	Citron.....	2.5 by 0.5	3
4	.....do.....	2.5 by 0.3	3
5	Sweet orange (Valencia).....	4 by 1.0	3
6	Sweet orange (navel).....	3 by 1.0	3
7	Sour orange.....	1.2 by 0.5	2
8	Controls.....	0	0

[1] 0=none; 1=slight; 2=medium; 3=copious.

The lesions here, though small at the time of observation, give some indication of differences in resistance. The common lemon was most affected, followed by the sweet orange (Valencia and navel) and the citron. Sour orange, as before, was quite resistant, though showing some effect, but was already beginning to form callus at the cut.

Experiments to test the effect of *Pythiacystis citrophthora* on small roots of different species of seedlings were also made.[8]

On November 15, 1915, mycelium of Pythiacystis and lemon fruits affected with Pythiacystis in different tests were placed on the healthy roots (with and without punctures) of the common lemon, sweet orange, pomelo, and sour orange. When last examined, on May 11, 1916, only the roots of the common lemon, where inoculated with *Pythiacystis citrophthora* (with punctures) and where inoculated with diseased lemon fruit (without punctures), were killed. The fungus was reisolated from the lemon root which had been inoculated with *P. citrophthora*. This experiment gives additional weight to the correctness of previous observations that even small and medium sized roots of the common lemon are frequently attacked by *P. citrophthora*, but that the small roots of other varieties are quite resistant.

Inoculations reported in other sections of this paper also give further indication of differences in resistance, especially as between the common lemon and sweet orange. The reasons for these differences in susceptibility is an interesting question in itself, which has not as yet been investigated. Our inoculation experiments would indicate that the differences in resistance in sour orange, sweet orange, and lemon, at least, can not be confined to the superficial layers of cells. If it were so limited the insertion of the parasite into cuts should cause equal effects in each variety.

MAL DI GOMMA IN RELATION TO PYTHIACYSTIS GUMMOSIS

Mal di gomma, due to *Phytophthora terrestria* Sherbakoff, is a gum disease with close relationships to Pythiacystis gummosis. It has previously been pointed out that certain phases of the Pythiacystis gummosis occurring on or near the main roots of sweet-orange trees are similar to those of mal di gomma, or footrot.

For this reason, certain footrot-like forms due to *Pythiacystis citrophthora* in California have previously been known by the name of mal di gomma according to Smith and Butler (*56*), 1908, and Fawcett (*26*). Since the name mal di gomma was first used in Florida to designate a common Florida gum disease, which is now known to be due to *Phytophthora terrestria*, it is proposed to restrict its use (in this country at least) to the disease due to this fungus.

This type of gum disease affects, for the most part, the bark on the lowest portion of the trunk and the upper portion of the first main roots, mostly below the surface of the soil. Gum usually forms on the trunk of the tree above the soil. The inner bark and finally the wood under-

[8] Boxes were constructed with one side consisting of glass plates inserted into grooves and covered by a removable wooden door fastened by hooks to hold it against the glass. After the young trees had become established in these boxes, the glass plate was removed to make inoculations on roots that had grown out against it and was then replaced in its former position. The assistance of Mr. E. E. Thomas, for whom similar experiments with Fusarium were made, is here acknowledged.

neath frequently develop a disagreeable fetid odor.[9] The bark dies and breaks away in patches, leaving bare, dead areas, which spread in all directions, but mostly downward, on the main crown roots and laterally around the trunk. Trees thus affected bear heavy crops of fruit and the leaves become yellow.

Phytophthora terrestria Sherbakoff (*53*) was first isolated by the writer from gumming lesions of an orange tree at Lindsay, Calif., in 1912, and was considered at that time to be only a peculiar strain of *Pythiacystis citrophthora*. Later, in 1914, it was isolated from mal di gomma lesions, as follows: (1) from a grapefruit tree at Palmetto, Fla. (*26, fig. 5*); (2) from orange trees in Cuba; and (3) from a tangelo tree on the Isle of Pines. The Florida and Cuban cultures were still considered to be strains of *Pythiacystis citrophthora* and were referred to as such in publications (*25, 26*). Stevens (*61*) later made an extended survey and isolated this species in a large number of mal di gomma cases from widely separated localities representing the principal part of the Florida Citrus belt. In the meantime, Sherbakoff (*53*) had described *Phytophthora terrestria* as causing rot of tomatoes in Florida. The cultures previously isolated at Lindsay, Calif., and in Florida and Cuba were then examined by Sherbakoff, who concluded that they were all the same species. A culture isolated by George Fawcett from a lemon tree in Argentina and sent to the writer in October, 1916, was also determined to be similar to this species. Later, the writer (*27*) determined that *Phytophthora terrestria* had markedly different growth-temperature relations from those of *Pythiacystis citrophthora*. The vegetative growth on certain culture media, the method of forming sporangia, and the effect on the host when inoculated, are so similar, however, as to make it seem probable that the two are closely related species. (See inoculation experiments, Tables XI and XII.)

Phytophthora terrestria is believed by some authors to be identical with *P. parasitica* Dastur. Ashby (*4*) has recently described a leaf-stalk rot of coconuts in Jamaica as being due to this latter fungus and states that Dastur has compared *P. terrestria* with *P. parasitica* and found them to be identical. Ashby has also found this fungus on tobacco and pineapple plants. Dastur (*19*) originally described his species as causing a disease of castor oil plants in India and later (*20*) found it attacking *Vinca rosea*. If *P. parasitica* and *P. terrestria* are the same species, a wide distribution of this fungus is indicated.

Comparative Inoculations with Phytophthora Terrestria and Pythiacystis

Many different series of inoculation experiments with *Pythiacystis citrophthora* and *Phytophthora terrestria* were made under varying conditions, the results of some of which are given in Tables XI and XII.

[9] This rotting of the wood, as well as the bark, and the accompanying "fetid odor" are believed to be due mainly to secondary organisms setting up fermentation and decay below the surface of the soil after killing of the bark by the primary organism. While gum may be formed below as well as above the surface of the soil, it is dissolved readily by moisture and is usually less conspicuous below the soil surface.

TABLE XI.—*Comparative effects of various "strains" of Pythiacystis citrophthora and Phytophthora terrestria on lemon and orange bark—Eureka lemon trunks about 6.5 cm. in diameter, budded high on sweet-orange stocks, at Riverside, Calif. Inoculations April 17, 1913; examination May 20, 1913*

Experiment No.	Source of culture.	Host and place on tree inoculation.	Killed area.	Gum formation.[1]
			Cm.	
1	*Pythiacystis citrophthora;* from lemon bark, Whittier, Calif.	Eureka lemon above bud union.	3 by 1.3...	2
2	.....do.....	Orange stock below bud union, same tree.	2 by 1.....	2
3	*Pythiacystis citrophthora* from lemon bark, San Diego, Calif.	Eureka lemon above bud union.	2.5 by 1.5..	2
4	.....do.....	Orange below bud union..	0.....	0
5	*Phytophthora terrestria* from orange bark, Lindsay, Calif.	Eureka lemon above bud union.	4 by 2.5....	3
6	*Phytophthora terrestria*, same.	Same tree on orange stock below bud union.	5 by 2.....	2
7	*Pythiacystis citrophthora* from lemon fruit, California.	Lemon bark above bud union.	30 by 18 (Pl. 6, C).	3
8	*Pythiacystis citrophthora*, same	Orange bark below bud union.	46 by 18 (Pl. 6, C).	2
9	*Phytophthora terrestria*, from pomelo bark, Palmetto, Fla.	Lemon bark above bud union.	20 by 5 (Pl. 6, E).	3
10	*Phytophthora terrestria*, same.	Orange bark below bud union.	25 by 9 (Pl. 6, E).	3
11	*Phytophthora terrestria* from orange bark, Cuba.	Lemon bark above bud union.	6 by 5 (Pl. 6, D).	2
12	*Phytophthora terrestria*, same.	Orange bark below bud union.	23 by 6 (Pl. 6, D).	3
13	*Phytophthora terrestria* from tangelo, Isle of Pines.	Lemon bark above bud union.	19 by 5 (Pl. 6, B).	3
14	*Phytophthora terrestria*, same.	Orange bark below bud union.	23 by 8 (Pl. 6, B).	2
15	Control (cut without inoculation).	Lemon and orange bark....	(Pl. 6 A)..	0

[1] 0=none; 1=slight; 2=medium; 3=copious.

TABLE XII.—*Comparative effects of different "strains" of Pythiacystis citrophthora and Phytophthora terrestria in inoculations on trunks of 20-year-old Lisbon lemon trees at Santa Paula. Inoculations, April 9, 1914; examination, August 14, 1914*

Experiment No.	Original source of cultures.	Organism.	Size of diseased area.	Gum formation.[1]
			Cm.	
1	Lemon tree, Santa Paula, Calif...	*Pythiacystis citrophthora.*	23 by 10....	3
	Lemon tree, Whittier, Calif.....	.....do.....	8 by 5.....	2
2	Orange tree, Lindsay, Calif.....	*Phytophthora terrestria.*	2.5 by 1.3..	1
3	Pomelo tree, Palmetto, Fla.....	.....do.....	8 by 4.....	2
4	Orange tree, Cuba.....	.....do.....	6 by 2.5....	2
5	Tangelo tree, Isle of Pines.....	.....do.....	10 by 5.....	2

[1] 1=slight; 2=medium; 3=copious.

The lesions produced by the inoculations with the two fungi were identical in general appearance. The characteristic manner of killing the bark, the formation of the outer noninvaded gummous zone, and the exudation of gum were the same with both fungi. Cuts without inoculation healed without producing disease.

In addition to these inoculations, a number of other comparative inoculations with pure cultures of *Pythiacystis citrophthora* and *Phytophthora terrestria*, using fruits affected with the two fungi, were made on the large main roots and at the crown of old orange trees. In nearly all the successful inoculations of this kind, lesions resulting from the two species of fungi could not be distinguished clearly one from the other and resembled true mal di gomma or footrot lesions as they occur in Florida. Most of the lesions became self-limited in three to four months on old orange trees, after enlarging to a maximum of 8 by 10 cm., but some made with *Pythiacystis citrophthora* progressed longer and developed large patches, in one case spreading 25 cm. and killing a large main root.

CONTROL OF PYTHIACYSTIS GUMMOSIS

More rational control methods based directly upon a knowledge of the cause and development of the disease and upon the results of many experiments [10] growing out of this knowledge now became possible. Previous control methods, though based largely on a different explanation of the cause, were partially successful because they frequently removed entirely the conditions contributing to infection and development of the disease, which conditions were formerly thought to be the sole cause.

The most successful method of prevention for new plantings is obviously the use of the resistant sour-orange stocks budded high. This method was employed in Italy after the orchards were killed out by gummosis and has since been used largely in Florida and California. To prevent gummosis on susceptible stocks or on low-budded trees, the method now in common use in California on heavy soils is to pull back the soil from the base of the trunk, thus exposing the top of the first main roots and making a circular ridge to exclude irrigation water from standing in contact with the trunk of the tree. As an added preventive on soils especially subject to gummosis, the base of the trunk is painted with Bordeaux paste or other noninjurious fungicide. In one experiment with Bordeaux used in this way on about 100 acres of lemons the number of new cases decreased from 123 in 1912 to 16 in 1914. By a similar treatment in another locality with 23,837 lemon trees the number of new gummosis outbreaks decreased from 727 in 1912 to 113 in 1914 (from 3.5 to 0.5 per cent). In a third experiment with 560 lemon trees, one-half of which was treated, 9 per cent of those receiving Bordeaux spray two previous years developed disease, against 21 per cent of those not so sprayed.

The treatment of trees after they are diseased consists in removal of the bark tissue from the invaded zone and the application of a fungicide to kill out the fungus in small bits of tissue possibly left behind and to prevent reinfection (Pl. 3, B–D). No attempt need be made to cut beyond the large outer gummous zone, since it has been shown that this does not contain the invading parasite and will recover rapidly after the inciting cause for the gumming has been destroyed.

[10] The detailed results of experiments in prevention and control will be presented in a bulletin from the University of California Agricultural Experiment Station.

PART II.—GUMMOSIS DUE TO BOTRYTIS CINEREA AND OTHER FUNGI

BOTRYTIS GUMMOSIS

INTRODUCTION

Botrytis gummosis differs from Pythiacystis gummosis, discussed in Part I of this paper, in that it causes softening of the invaded bark in the early stages and produces conidiophores and spores in damp, cool weather. In the later stages the outer layer of bark is killed and becomes dry and hard much in advance of the inner layer, while there is a greater tendency than in Pythiacystis gummosis for the tree to renew the bark underneath the dead hard layer and there is usually also a less copious flow of gum. Botrytis gummosis is confined in California almost exclusively to lemon trees growing in the coastal regions and is usually seen on trees more than 10 years of age. This disease should not be confused with a desquamated-bark (shellbark) condition in which the outer bark on old lemon trees dies, cracks, and breaks away in longitudinal strips, a condition which is somewhat similar to that frequently brought about in the later stages of Botrytis gummosis. Neither disease should be confused with psorosis (scaly-bark) of sweet-orange trees.

The causal fungus, *Botrytis cinerea* Lk., depends to a much greater extent than does *Pythiacystis citrophthora* Sm. and Sm. on wounds or other conditions predisposing the bark to attack.

The writer's attention was first called to this type of gummosis early in February, 1912. After a period of moist, cool weather, patches of bark 15 to 30 cm. long and half as wide presented the gray furry appearance characteristic of the fruiting bodies of *Botrytis cinerea*. In a later survey of the Citrus districts of California, *Botrytis cinerea* was usually found associated with this type of gummosis and was isolated from a large number of diseased lesions. *Penicillium roseum* Link,[11] *Fusarium* sp., and several other fungi were also frequently conspicuous.

The important question at once arose, whether any of these fungi might break down sound tissue and initiate conditions leading to disease and gum formation or whether gumming and associated death of the bark might result primarily from injuries or other contributing conditions, the fungi playing only a minor or secondary part, as had previously been assumed. It seemed advisable, therefore, to attempt to answer this question experimentally.

INOCULATION EXPERIMENTS

Preliminary Tests

On March 7, 1912, at Santa Paula, a preliminary set of rough inoculations was made with diseased tissue and with fungi taken directly from the surface of the bark.

Sixteen Lisbon lemon trees 18 years old, on sweet-orange stocks, were used for the inoculation. The portion of the bark to be inoculated was washed first with water and then with alcohol, and the flame of an alcohol lamp was quickly passed over the surface. A cut about 3 cm. long was made through the bark with a heavy sterilized knife. Into these cuts the materials for inoculation were inserted. The cuts were made at varying

[11] Determination of this species was kindly made by Dr. Charles Thom.

distances, ranging from 5 to 60 cm. above the "bud union." With every inoculation a similar cut, to serve as a control, was made in the opposite side of the same tree. A sheet of paraffined paper was then tied against the surface of the bark over each cut and fastened with wax at the upper edge to exclude rain and dust.

The different kinds of inoculum and the number of trees were as follows: (1) Small pieces of sound bark and wood, four trees; (2) bit of diseased bark, two trees; (3) small bits of wood permeated with gum from outer gummous zone, two trees; (4) small portion of exuded gum, one tree; (5) bits of dead bark containing *Penicillium roseum* Lk., two trees; (6) hyphae and spores of *Botrytis cinerea*, taken from rotting diseased bark, three trees.

None of the inoculations with sound wood or bark, gum-filled wood from the outer gummous zone, or exuded gum produced any gumming or development of disease. All the inoculations from diseased tissue, however, showed gum exudation within 2 to 4 months, and in one case a lesion 8 by 15 cm. developed in 9 months. One of the inoculations with *Penicillium roseum* showed slight gum exudation in 4 months without development of a lesion, but the other cut healed without gumming. All three of the Botrytis inoculations resulted in gummosis of the same type as that from which the fungus was obtained. In one of them slight softening of the bark was noted in 10 days. A softened area of bark which measured 8 by 2.5 cm. in 50 days (with fruiting of Botrytis on the surface) had increased to 10 by 4 cm. in 4 months, with copious exudation of gum some of which was exuding through cracks 50 cm. directly above the cut in the outer noninfected gummous zone. About 1 year after this inoculation the space over which the bark was dead to the wood measured 10 by 2.5 cm., and this was surrounded by an irregular area over which only the outer bark was killed, making the entire area 30 by 27 cm. Botrytis was fruiting at various places over this area.

A second set of experiments was then carried out. These were planned to test the influence of various kinds of wounds, the influence of obstructions in the sap current, and finally, the influence of continued pressure upon the bark. These experiments were made in July, 1912, on sound trunks of 19-year-old Lisbon lemon trees at Santa Paula. The following methods were used both with and without contamination with pure cultures of *Botrytis cinerea:* Cuts vertically or horizontally through the bark, auger holes with and without glass or wooden plugs, bruises made by light and heavy blows from hammers, wounds made by slicing off both thin and thick layers of bark, wounds made by entirely cutting off large areas of the bark and so forth. Constant pressure was also exerted against the bark with wooden blocks tightened by means of screws in an iron collar.

It was difficult to make these inoculations or injuries in tree trunks in the open and keep them absolutely free from organisms. In attempting to overcome this difficulty the following method was adopted for the most important of these experiments. A cloth hood to be tied to the tree trunk was made by fastening a piece of fumigation-tent cloth to a wooden barrel hoop severed on one side. Strings were fastened to the cut ends of the hoop and to the corners of the cloth opposite the hoop. The hoop was allowed to hang down. The upper strings were tied to the trunk of the tree just below the branches and the hoop was fastened

below so as to cause the cloth to flare outward and thus protect the wound from falling dust and excessive currents of air. In order to prevent dust in dry weather, the under surface of the hood, the trunk, and the soil around the base of the tree were sprayed with water. The area of bark to be experimented on was washed, first with water and then with alcohol, and quickly flamed with an alcohol lamp. The augers, hammers, knives, or other instruments used were sterilized either by heat or by alcohol which was allowed to evaporate from their surfaces. Either grafting wax or glass slides sealed with putty were used as a covering in most of the experiments.

There was no gum exudation or development of disease in connection with any of the experiments with which attempts were made to keep the bark free from contaminations. With the experiments in which spores of *Botrytis cinerea* were used as a contamination, however (except where the bark was not injured) gum exuded, and in most cases typical Botrytis gummosis lesions developed around the place of injury.

The results of these experiments appeared to show that injuries were not sufficient in themselves to induce gum diseases. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue were readily produced.

Inoculations with Pure Cultures of *Botrytis cinerea*

Many inoculations have been made with pure cultures of *Botrytis cinerea* and the following is typical of the results.

On July 12, 1912, spores and sporophores of *Botrytis cinerea* were inserted in a cut 2.5 cm. long, on the trunk of an 18-year-old lemon tree at Santa Paula. The wound was then covered with oiled paper. Gum was exuding rapidly by July 20 and continued to do so until a diseased area 4 by 5 cm. was noted on August 22. On November 21, there was a softened, dead area of bark, 15 by 5 cm., which on February 14, 1913, was 23 by 8 cm. in size and on June 27, 1913, 28 by 10 cm., with new gum, and Botrytis fruiting on a part of the surface. On July 28, 1913, the main area was 32 by 10 cm., and a number of smaller areas were scattered over the same side of the trunk. On scraping the trunk for treatment at this time, it was found that only a small area of bark was killed through to the wood, only the outer cortical layer of the remaining part being dead. Bordeaux paste was applied to one lateral half of the scraped portion. On November 25, 1913, the disease was seen to be arrested on the portion treated with Bordeaux, but further dying of outer bark had taken place on the other portion. A similar cut without inoculum made on the opposite side of same tree healed without gumming or any other apparent effect.

In all, about 40 inoculations with pure cultures of *Botrytis cinerea* were made on lemon trees, most of which resulted in gummosis of the type represented by the previous example. The general results of a number of these are given in Table XIII.

TABLE XIII.—*Inoculations with pure cultures of Botrytis cinerea, on 18-year-old lemon trunks except where noted*

Experiment. No.	Date of inoculation.	Host.	Gumming.[1]	Botrytis fruiting.[1]	Cracked outer bark area.[1]	Date of examination.	Area killed, outer bark.
							Cm.
1	July 12, 1912	Lemon	x	x	x	July 28, 1913	32 by 8
2	.....do.....	...do...	x	o	x	Mar. 9, 1916	30 by 8
3	Aug. 23, 1912	...do....	x	x	x	.....do.....	30 by 8
4	Dec. 23, 1912	...do....	x	x	x	.....do.....	10 by 8
5	Feb. 20, 1913	...do....	x	x	x	.....	
6	Mar. 14, 1913	...do....	x	x	x	Sept. 3, 1914	20 by 5
7	July 13, 1913	...do....	x	o	x	Apr. 7, 1914	Very small.
8	.....do.....	...do....	x	o	x	.....	
9	.....do.....	Orange .	o	o	o	Feb. 6, 1915	No effect.
10	.....do.....	Lemon..	x	.....	x	Apr. 2, 1914	Very small.
11	.....do.....	...do....	x	.....	x	.....do.....	Do.
12	.....do.....	...do....	x	.....	x	.....do.....	Do.
13	.....do.....	...do....	x	.....	x	.....do.....	Do.
Control cuts for each experiment.....			o	o	o		

[1] x=positive; o=negative.

With most of these inoculations made on old lemon tree trunks such effects as the softening and death of the bark through to the wood in the earlier stages, the appearance of fruiting bodies, formation of gum, and the subsequent development of an area of dead outer bark surrounding the initially invaded area and finally of an outer noninvaded gummous zone were found to be identical with those of cases of the disease occurring naturally.

Further inoculations into cuts on young bark of several species of Citrus and of deciduous trees are reported in Table XIV.

TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts*

I. INOCULATED JULY 2, 1913; EXAMINED AUGUST 11, 1913

Experiment No.	Host.	Kind of tissue.	Results.
1	Valencia orange...	Young branch.....	Gumming slightly.
2	Sour orange.....	Small branch.....	Healing without gumming.
3	Lemon.....	Hardened bark.....	Cut filled with gum and healing back of it.
4	.....do.....	Young twig.....	Gum more copious than last; not healing.
5	.....do.....	Same as above without inoculum.	Healing without gumming.
6	Almond.....	Stem of young tree.....	Gumming with small portion of bark killed at cut, then healed.
7	Peach.....	.....do.....	Much gumming. Killed slightly on each side of cut. Bark area 6 cm. long in wood.
8	Plum (Burbank)..	.....do.....	Gumming at first and then healing.
9	Pear.....	.....do.....	Healed with enlarged scar.
10	.....do.....	Same as above without inoculum.	Healing perfectly.

TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts*—Continued

II. INOCULATED APRIL 17, 1913; EXAMINED MAY 20, 1913

Experiment No.	Host.	Kind of tissue.	Results.
11	Common lemon...	Trunk 3 years old.....	Bark soft and decaying over areas of 0.5 by 1.5 inches. Much gum exuding.
12	Sweet orange.....	.....do.....	Bark slightly killed. Slight internal gumming.
13	Same as above without inoculum.	.....	No effect on bark.

III. INOCULATED FEBRUARY 6, 1915; EXAMINED MAY 18, 1915

14	Common lemon...	Branch about 2 years...	Gumming and fruiting of Botrytis on killed bark.
15	Rough lemon....	Trunk 2 years old.....	No effect.
16	Sour orange.....	.....do.....	Do.
17	Same as above without inoculum.	.....	Do.

With comparatively young common lemon, peach, and almond trees these inoculations with spores placed in cuts resulted in the formation of considerable gum. The gum was slight in sweet orange and plum. No gum was induced in sour orange, rough lemon, or in any of the control cuts except on peach. The death of the bark was slight and unimportant in extent except in the common lemon and peach.

In general the results of all these experiments showed that the effects of *Botrytis cinerea* were quite different from those of Pythiacystis gummosis. This fungus, unlike *Pythiacystis citrophthora*, was not able to make an entrance except through some wound or defect in the bark, and it was not able to progress so rapidly in killing the bark through to the wood. After a limited portion was killed to the wood, however, a larger surrounding area was involved, in which only certain outer layers of bark tissue were killed, leaving the cambium alive and capable of renewal. There was also in the Botrytis gummosis an outer gummous zone beyond the invaded zone, but this was usually less extensive and less rapidly formed than in Pythiacystis gummosis. Other conditions being equal, there was usually somewhat less gum formation in Botrytis gummosis than in Pythiacystis gummosis.

The fungus was reisolated from the softened invaded area of a large number of these lesions. Attempts to isolate the fungus from the outer gummous zone failed, just as they did in Pythiacystis gummosis. Only rarely was Botrytis isolated from the area where the outer bark was dead and hard. Cultures showed that this outer dead cortical layer following Botrytis inoculations is, under such conditions as prevail at Santa Paula, rapidly occupied by species of Alternaria, Cladosporium, Penicillium, Colletotrichum, Fusarium, and other fungi as well as bacteria.

FACTORS FAVORING BOTRYTIS GUMMOSIS

Many contributing conditions favor the occurrence and the severity of this disease. These are similar to those which favor Pythiacystis gummosis, mentioned in Part I of this paper, to be discussed in more

detail in a subsequent bulletin of the California Agricultural Experiment Station.

Injuries of various kinds to the bark, not only near the soil but anywhere on the trunk or large branches, may lead the way to infection and development of Botrytis gummosis when the conditions of moisture and temperature are also favorable. This disease is frequently severe on living tissue of trees that have been injured by frost. The fungus may become established in such trees first in a small portion of dead or dying tissue and then advance rapidly into tissue which appears to be sound.

A desquamated condition of bark, fairly common on old lemon trees in the California coastal regions, is also frequently accompanied by Botrytis gummosis. It furnishes dead outer bark tissue from which the fungus may advance. This desquamated condition is similar in appearance to that which usually follows inoculations with *Botrytis cinerea* on sound tree trunks and with which it is often confused, but which is thought to be due to other causes.

The previous use of "neat's-foot oil" in the treatment of gummosis also encouraged the growth of this fungus. Lemon trees previously treated at Santa Paula by scoring the bark and painting with neat's-foot oil were observed in February and March of 1912 to have their trunks fairly well covered with a gray coating consisting of the sporophores and spores of Botrytis. The bark on these trees was found to be in various stages of soft decay with the exudation of large masses of gum. Experiments also showed that this fungus grew better on bark treated with neat's-foot oil either before or after infection by the organism than on bark free from this oil. More recently the application of neat's-foot oil to Citrus trees has been largely given up, and the more severe stages of this disease, such as were previously seen, have not been observed.

CONTROL

The principles governing the control of this type of gum disease are similar to those of Pythiacystis gummosis: (1) The prevention of infection by avoiding injuries, and the use of fungicides, and (2) if that is not done, the elimination of the invaded tissue. As the result of many different experiments in which growers took a prominent part, a method consisting largely of scraping off the outermost layers of bark proved to be best adapted for treatment of this disease. The portion where the bark is totally killed is cut away, but beyond this, where only the outer layers of bark are dead, these outer layers only are scraped off, leaving the live inner layer next to the cambium intact. To prevent further invasion of bark it is usually found necessary to scrape the sound bark several inches beyond the margin of the affected region. A sharp curved tool made on the principle of a box scraper is in general use for this purpose. Where both types of gummosis are present on the same trees, as is frequently the case, this method is applicable to the combined lesions produced. The cut or scraped portions are then painted with a fungicide. Bordeaux paste, and some of the coal-tar products which contain only the heavier oils, have given good results in experimental work and in practice. A discussion of the experiments on which these control methods are based will be reported in a bulletin of the California Agricultural Experiment Station.

SCLEROTINIA GUMMING, DUE TO SCLEROTINIA LIBERTIANA

This disease, usually of minor importance, occasionally has been found associated with rapid dying of bark on the roots and trunks of Citrus trees growing in damp, cool situations, especially after periods of severe frost. The bark is at first soft, as if from an attack by *Botrytis cinerea*. Though the fungus usually advances more rapidly than Botrytis, it is soon checked, and callus begins to form as soon as gum accumulates. Later, as the bark dries, it is left in shreds and large black sclerotia are found within and under this bark. Its effect on Citrus twigs has been described by C. O. Smith (*54*), who refers to the gumming usually accompanying its attack. It appears to infect the young growth, usually at the blossoming period and frequently extends back into larger branches.

When found on the trunk or roots, observations have indicated that previous injury of the bark was usually necessary for the entrance of the fungus. It has frequently been found on young trees following frost injuries, apparently advancing from frost-injured tissue into tissue not killed by frost. It has been observed on a 20-year-old lemon tree where all the roots had been infected, probably from injuries made in digging about them and placing vetch straw near the crown in damp, cool weather. An old seedling orange tree was also observed with the bark on one side of the trunk killed by the fungus, which had apparently gained entrance through a slight sunburned area and had advanced into the live bark for some distance.

In order to obtain some idea of the ability of *Sclerotinia libertiana* to break down sound bark and induce gumming, several inoculations with pure cultures were made on healthy lemon trees, of which the following examples may be given:

On February 20, 1913, a bit of mycelium from a pure culture was inserted into a vertical cut in the bark of an 18-year-old Lisbon lemon tree. A similar cut to serve as a control was made on the opposite side of the same tree. These were then covered with oiled paper. The cut not inoculated healed normally, but the bark adjacent to the inoculation was soft on one side within 4 weeks. In 8 weeks there was a dead, soft area 15 by 8 cm., which had increased to 22 by 9 cm. in 11 weeks with a white mycelium conspicuous over a smaller area of 13 by 5 cm. About 4 months after inoculation (June 27, 1913) the area of dead bark measured 30 by 14 cm. and increased to 45 by 15 cm. by July 28, 1913, after which it soon ceased enlarging and became self-limited. The gum began to appear in 6 weeks; its rate of formation increased rapidly and reached a maximum about 3 months after inoculation. It ceased to increase about 5 months after inoculation. The sclerotia were seen to have formed in flat plates under and within the bark in 4 months and appeared to be alive for at least 2 years, but no subsequent activity was apparent in this lesion for a period of at least 3 years. The killed bark showed the characteristic shredded appearance. Without treatment, there was a complete stoppage of further invasion by the fungus.

A 2-year-old lemon tree was similarly inoculated 1 inch above the bud union on February 25, 1913. By May 7, 1913, the tree was girdled and showed gumming, the bark being killed 6 inches upward and 1 inch downward to the bud union. The invasion did not advance into the sour-orange tissue of the stock. The foliage at this time showed no effect, but by June 27, 1913, the leaves were wilted. In both these cases similar

cuts not inoculated, made as controls, healed without visible injury to the tree.

As indicated by these inoculations, the attack of *Sclerotinia libertiana* may be very severe and its progress very rapid for a comparatively short time, and then it may be quickly halted. If the tree trunk attacked is small, girdling and death may result, while on a large trunk with not more than one point of infection, self-recovery may take place. Observation shows that the halting of the invasion is usually coincident with the formation and exudation of considerable quantities of gum.

EXPERIMENTS TO TEST THE POSSIBLE RELATION OF OTHER ORGANISMS TO GUMMOSIS

A number of other organisms, most of which were found commonly on dead or decaying bark of Citrus trees, were used in inoculation experiments to ascertain their relation, if any, to gummosis.

The results of inoculations with various cultures of *Fusarium* sp., commonly found associated with Pythiacystis gummosis, have already been presented on page 205. The effect of Fusarium alone was insignificant. The death of the bark at the inoculated cuts was inconspicuous, and only slight death of the outermost layer of bark resulted over small areas. Only part of the inoculations produced gum, usually in small quantity.

Penicillium roseum was frequently found on dead bark affected with gummosis. The results of some of the inoculations with pure cultures of this fungus are presented in Table XV.

TABLE XV.—*Inoculations with Penicillium roseum at Santa Paula*

Experiment No.	Date of inoculation.	Kind of inoculation.	Gum.	Initially killed bark at inoculation point.[1]	Cracking of outer bark.[1]
1	July 12, 1912	Vertical slit 2 cm. long, covered with oiled paper	2	1	2
2	Aug. 23, 1912	Vertical slit	2	1	0
3	Feb. 21, 1913	Vertical slit not protected	2	1	2
4	.....do.....	Controls on same trees as above	0	0	0
5	Feb. 20, 1913	Vertical slit not protected	1	1	0
6	.....do.....	Control on same tree	0	1	2

[1] 0=none, 1=slight, 2=medium.

Gumming was induced in all the cuts inoculated with *Penicillium roseum* in Table XV. The dead bark adjacent to the inoculated cuts was slight in amount. The outer layer of bark surrounding two of the inoculated cuts and only one of the controls died and cracked.

Inoculations were made with a number of other organisms, as shown in Table XVI. All of these were found on Citrus trees except *Coryneum berynkii* and *Pseudomonas cerasius*.

TABLE XVI.—*Inoculation with miscellaneous organisms*

Experiment No.	Date of inoculation.	Variety and age of host.	Organism.	Gumming.[1]	Death of bark.[1]
1	Nov. 23, 1912	Lemon, 19 years..	*Alternaria* sp. from injured fruit.	1	1
2	Feb. 25, 1913	Lemon, 2 years....	*Alternaria* sp.....	0	1
3	May 24, 1912	Lemon 16 years...	*Coprinus atramentarius*, mycelium.	0	0
4	.....do.....	.....do.....	Spores of same.....	2	1
5	July 13, 1913	Lemon, 19 years...	*Coprinus atramentarius* mycelium.	2	1
6	May 24, 1912	Lemon, 16 years...	*Hypholoma* sp. spores...	1	0
7	Aug. 3, 1912	Lemon, 16 years (2 trees).	*Hypholoma* sp. mycelium.	1	0
8	Sept. 25, 1912	Lemon, 19 years...	*Cladosporium* sp.....	0	0
9	.....do.....	.....do.....	*Rhizopus* sp.....	0	0
10	Sept. 4, 1913	Lemon, 18 years...	*Penicillium digitatum*...	0	0
11	Aug. 3, 1912	Lemon, 16 years...	*Diplodia* sp.....	2	1
12	Nov. 23, 1912	Lemon, 19 years...	*Spegazzinia ornata*.....	0	0
13	July 13, 1913	.....do.....	*Coryneum berynkii* from peach.	2	1
14	.....do.....	Orange stock, same tree.	.....	1	0
15	July 12, 1912	Lemon, 19 years...	*Pseudomonas cerasius* Griffin.	0	0
16–25	.....do.....	.....do.....	10 different cultures of bacteria inoculated from bark killed by gummosis.	0	0
Controls, without inoculum to correspond with each of the foregoing experiments.				0	0

[1] 0=none, 1=slight, 2=medium.

The results show that several different organisms are usually capable of inducing the formation of a small quantity of gum and a limited amount of injury to cells adjoining a wound when inserted into cuts on sound tissue but are without any noticeable effect in producing definite diseases. This gum formation or death of tissue was, in these cases, as well as with *Fusarium* sp., and *Penicillium roseum*, insignificant in amount as compared with that produced in cases of either Pythiacystis or Botrytis gummosis.

PART III.—GUM FORMATION AND ITS RELATION TO THE DEVELOPMENT OF DISEASES

INTRODUCTION

In Parts I and II of this paper the relation of certain fungi to definite diseases in which gum formation was a conspicuous feature has been discussed. It was shown experimentally that the most destructive types of gum diseases on Citrus in California in which there is progressive dying of tissue over large areas are due to fungus invasion. Fungi, however, are not necessary to mere gum formation, since it has been shown that other agencies, such as chemical injections, may induce gum formation in the absence of microorganisms.

It is the purpose of Part III to discuss more especially the process of gum formation itself, the conditions facilitating its formation, and its relation to the development of diseases.

NATURE AND ORIGIN OF THE GUM

Although gum appears to be formed in many other plants continuously as a "normal" process, it is usually not formed in Citrus except under the influence of stimuli more or less injurious to the tree. Citrus gum is similar to cherry gum and gum arabic, the latter of which is known to retain the nature of an acid, the molecule being composed of a number of sugar residues grouped about an acid nucleus in such a way as to leave the acid group exposed.

These gums differ from resins in being for the most part soluble in water and insoluble in alcohol, while the opposite is true of the resins. This solubility in water also distinguishes them in a rough, imperfect way, from mucilages, which have a more slimy consistency and merely swell up in water. There are, however, all gradations between gums so defined and the mucilages. As to origin and chemical composition, the distinction between the vegetable gums and some of the mucilages can probably not be clearly maintained. These exuded gums, however, should probably be distinguished from a hard, vitreous gumlike substance known as wound gum, which Higgins (*40*) refers to as occurring in the wood elements in the vicinity of wounds as a general phenomenon in woody plants. It differs from the ordinary gums in not swelling in water and in giving the lignin test.

Although there has been considerable difference of opinion as to the direct origin of gums and mucilages in plants, most investigators, including Grafe-Wien (*35*), Czapek (*18*), and Lloyd (*45*), have concluded that the gums like those represented by gum arabic, cherry gum and Citrus gum, are derived mainly from the cellulose walls. Greig-Smith (*36*), however, has reported the formation of gums of this nature by the direct action of bacteria on certain culture media containing no cellulose. Gum related to the dextrins also has been obtained by the action of *Bacillus radicicola* on culture media containing sacchrose by Buchanan (*12*), who concludes that this gum arises from the diffluent wall of the bacterial cell. MacDougal, Richards, and Spoehr (*47*) have pointed out that in cacti the formation of mucilagelike gum results from the dehydration of sugars and condensation of their products as a normal process in this plant.

In Citrus, however, the gum appears to arise mainly from the hydrolysis of the cellulose walls. The initial gum originates, according to Butler (*13*) and Floyd (*31*), between the medullary rays usually in thin-walled xylem cells newly laid down. The protoplasm becomes more granular, the cells round out, separate from each other, and dissolve from the outside inward, and the space is finally occupied by a mass of gum. It may also be derived apparently from the breaking down of other and older tissues in connection with some of the severe types of Citrus gummosis.

PHYSICAL EFFECTS AND GUM FORMATION

MECHANICAL INJURIES

The writer has not been able to induce gum exudation on healthy Citrus trees by mechanical injuries alone, provided these injuries are

kept clean and reasonably free from contamination with microorganisms or unusual chemical substances.

A number of experiments were carried out to test (a) the influence of various kinds of wounds, (b) the influence of obstructions in the sap current by insertion of substances such as glass or wooden plugs, and finally (c) the influence of continued pressure upon the bark. Most of these experiments were performed with and without contamination with spores from pure cultures of *Botrytis cinerea* and are shown in Table XVII.

TABLE XVII.—*Experiments with injuries with and without contamination, started in July, 1912, on sound trunks of 18-year-old lemon trees at Santa Paula*

Treatment of tree.	Number of experiments.	
	Not inoculated.	Inoculated.
Tangential slice of outer bark cut off and microscopic slide fastened over with putty	2	2
Surface of bark lightly scraped and covered as above	.....	1
Bark not injured but covered as above	1	1
Long slits made through bark and covered with grafting wax	1	1
Areas of bark about 8 by 4 cm. cut away and covered with cold liquid grafting wax	5	5
Bark on trunk injured with heel of heavy boot, as if climbing tree	.....	1
Bark injured by throwing wire coal basket against trunk	.....	1
Bark injured by blow from blacksmith's hammer and covered with wax	2	2
Auger holes 1.3 to 2 cm. deep and 1 to 1.5 cm. in diameter filled with glass tubes and sealed in with wax	5	2
Pressure exerted against wooden blocks, on opposite sides of tree, by means of screws in an iron collar	3	.....

On none of the injuries shown in Table XVII, kept uninoculated, was there any gum exudation or development of the disease. All such injuries healed in the usual way. With all the experiments in which spores of *Botrytis cinerea* were used as a contamination, however, except where the bark was not injured and one of those with glass tube used as a plug, gum exuded and in most cases typical *Botrytis gummosis* lesions developed around the place of injury.

The results of these experiments appeared to show that injuries in themselves were not sufficient to induce gum formation. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue readily took place. Other injuries of various kinds also failed to induce gumming in most cases even when no means were provided to keep the wounds free from chance contaminations from the air or from water during rains. These same wounds, however, when purposely infected with *Botrytis cinerea* or other injurious organisms resulted in gum formation. Mechanical pressure and obstructions placed in the conducting system likewise failed to induce gum in these lemon trees. In previous experiments conducted by Fawcett (*22*), clean injuries made by cutting through the bark of *Prunus persica*, *Prunus umbellata*, *Prunus serotina*. *Laurocerasus caroliniana*, *Xanthoxylum americanum*, and *Rhus glabra* in Florida failed to induce gum, but when similiar injuries in all these species were inoculated with *Diplodia natalensis*, gum formation resulted.

In Prunus and other plants, however, mechanical injuries in themselves have been considered as sufficient to induce gum formation. Butler (*13*) was successful in inducing gum formation on peach, cherry, and plum by bruising the bark with a mallet. No mention is made of similar experiments on Citrus.

The injuries on Citrus made by a number of insects are frequently followed by gumming, usually slight in amount. Small drops of gum may form on fruit at points of injury produced by the orange tortrix (*Tortrix citrana*), and on small tree trunks and limbs by grasshoppers, katydids, and other insects. To what extent this gumming may be due to secretions of the insects and to what extent to the entrance of microorganisms at the time of injury is uncertain. Our negative results from mechanical injuries on Citrus kept sterile and free from chemical stimuli would indicate that this gumming was probably not due to the injury or wound in itself.

BURNING

In the author's experience, burning was not in itself any more effective than mechanical injuries in producing gum formation in Citrus. The flame of an alcohol lamp was held against the trunk of young Citrus trees until the bark was severely injured, but no gumming resulted. Examination of many Citrus trees that have been accidentally injured by fire has shown that bark may be killed on one side of twigs and branches without resulting in gum formation. Severe injuries from sunburning on Citrus have also been observed to be free from gum formation, provided they are not followed by invasion of parasitic or wood-rotting organisms. Butler (*13*), however, reports the production of gum on young shoots of Prunus by burning with a hot iron.

FREEZING

Freezing has also been considered a frequent cause of gum formation. Observations of hundreds of Citrus trees in California in all stages of injury from frost have not indicated that freezing in itself is an important factor in initiating gum formation. Frost injury, however, may frequently be followed by invasion of organisms such as *Botrytis cinerea*, *Sclerotinia libertiana*, or other fungi, which after becoming established in the injured tissue may advance rapidly and induce gumming in tissue apparently sound.

DESICCATION

Drying or partial desiccation of cells in plants has been suggested as an important factor in gum formation. As the result of a number of controlled experiments with species of Prunus, drying of the tissue was considered by Higgins (*40*) as an important factor in the acceleration of gum formation. Sorauer (*59*, *p. 708*) cites Martin as mentioning the action of dry desert winds of autumn and winter as having a relation to gum formation in *Acacia senegal*. It has been pointed out by MacDougal, Richards, and Spoehr (*47*) that when Opuntia plants are subjected to long periods of drought, resulting in partial desiccation, the sugars, which have a low water-holding capacity, are converted into the pentosans or mucilages, which have a high imbibition capacity.

That this factor of partial desiccation operating alone can not account for excessive gum formation in Citrus seems to be indicated by the fact that such gum formation does not necessarily follow injuries from frost

or sunburn, nor does it necessarily result from drying of tissue in wounds if these are kept uncontaminated by microorganism or chemical stimulants.

Some of the results of the investigations reported in Parts I and II of this paper have a bearing on this subject. It was shown that gum formation could be induced in cuts inoculated by *Pythiacystis citrophthora* or *Botrytis cinerea* where these were covered with grafting wax or waxed paper, which presumably prevented undue loss of water from the surface. It is, of course, possible that dehydration of the cells invaded by the parasite might have taken place even in this case, without loss of water through the surface, by changes in internal conditions by which water was lost to the surrounding tissues. With Pythiacystis gummosis excessive water in the soil about the trunks, rather than drying or partial desiccation, is the condition favorable for the invasion of the bark by the causal organism that is instrumental in bringing about gum formation.

While partial desiccation in itself does not appear to be an important factor in initiating gum formation in Citrus, it is probably one of the factors in producing an acceleration of the process when the other necessary factors are already in operation. It has been noticed also that when a lesion is developing and gumming moderately, the coming of dry weather is frequently followed by increased exudation of gum for a brief period. If the weather is sufficiently dry, however, the enlargement of the invaded zone often ceases, probably due to the dying out of the parasite, and the rate of gum formation soon decreases to zero. It appears that in Pythiacystis gummosis, at least, the conditions of desiccation that tend to increase the gum flow are frequently those that hinder or kill out the causal agent, while furnishing a temporary stimulus to increased gum formation. If, as seems highly probable, partial desiccation of the cells is one of a group of factors which favor excessive gum formation in Citrus, it seems clear that other more important influences or stimuli must precede or accompany it.

CHEMICAL STIMULI AND GUM FORMATION

Many substances have been reported as inducing gum formation in Citrus. A study of gum formation was made by Floyd (*31*) by the introduction of 28 organic and inorganic substances into young Citrus trees. Thirteen of these induced gum formation. Most of them were acids, alkalies, or salts of heavy metals. In general, the salts of the heavy metals brought about the greatest amount of gumming. The gum formation was coincident with the injury from the chemicals; the gum was small in amount and was formed in proximity to the region of insertion of the chemicals except in case of some of the salts of the heavy metals. The gum originated in all cases in the live embryonic xylem tissue in regions or zones beyond the dead area produced by the chemical. None of the cuts or injuries used as controls, and not inoculated with chemicals, produced any gum formation. The dead area produced by the chemical is thus seen to be directly comparable to the invaded zone in Pythiacystis gummosis, and the region of chemically induced gum formation beyond this is directly comparable to the noninvaded "outer gummous zone" of the disease.

Experiments on Citrus had been made previously with a limited number of chemicals. Butler (*13*) induced gum formation with sul-

phuric, phosphoric, nitric, and lactic acids, and with potassium hydrate but failed to induce it with acetic acid or kerosene; and Fawcett (*23*) induced gum with nitric, sulphuric, acetic, citric, and phosphoric acids, copper sulphate, mercuric chlorid, and ammonium lactate but failed to induce it with carbolic acid.

Among the chemical stimuli that have been seen to result in gum formation occasionally in Citrus orchards may be mentioned: (1) liquid hydrocyanic acid spilled on the soil too near the roots of trees; (2) hydrocyanic acid gas used in fumigation; (3) spray mixtures containing copper sulphate not properly neutralized with lime, or containing other toxic substances; (4) ant poison containing arsenic.

It has not been possible, however, to duplicate entirely by chemical stimuli any of the typical maladies produced by certain organisms. While the gum formation induced by chemicals may be slight or extensive, the stimulus is soon at an end and the wound usually begins to heal promptly without the long-continued, progressive killing and gumming characteristic of Pythiacystis and Botrytis gummosis. Much confusion and apparent differences in results regarding gummosis have arisen from a failure to distinguish between the severe types of gummosis induced through the agency of microorganisms and those more temporary and usually milder types brought about by chemical or other stimuli. While the nature of the gum exuded may be the same, the manner in which the accompanying injury develops is usually very different.

ENZYMS AND GUM FORMATION

Only a small amount of experimental work on the rôle of enzyms in Citrus gummosis appears to have been done. The results of the writer's experiments will be presented here merely as suggestive of certain possibilities in connection with this subject.

Savastano (*52*) concluded that in the form of Citrus gummosis due to *Bacterium gummis*, a toxin secreted by the organism, spreads out considerable distances beyond the invaded tissue, stimulating the gum formation. In line with this view, Higgins (*39*) in the study of plum wilt, concluded that the fungus *Lasiodiplodia triflorae* Higgins secreted a toxic substance which reacted directly or indirectly on the zymogen of the host cells and brought about the production of a gum-forming enzym. Butler (*13*), on the other hand, had concluded that enzyms had no part in gum formation in Citrus or Prunus. This view, it seems, was based mainly on the origin and histology of the gum pockets and not on microchemical tests or experiments to detect the presence of enzyms. (See criticism of this view by Wolf) (*65*). Floyd (*31*) made certain microchemical tests with material taken from gum pockets on Citrus branches affected with exanthema and concluded that the enzyms present were probably hemicellulase and pectinase.

To get definite information as to the possible influence of enzyms or other filterable substances on gum formation in Pythiacystis gummosis, the following experiments were carried out.[12]

EXPERIMENT 1.—On October 1, 1912, lemon bark containing Pythiacystis gummosis lesions was cut from two large trees at Whittier, Calif., and 350 gm. of green material were ground, first in a meat grinder, then

[12] The assistance of H. D. Young, formerly of the Southern California Pathological Laboratory, in carrying out these experiments is acknowledged.

in a mortar with sand and water. One liter was decanted off and filtered through a sterilized clay filter. No organisms were found in the filtrate. Part of this filtrate was boiled and the other part left unheated. Two small holes slanting downward were bored, one in each side of a large lemon tree, and burettes holding about 50 cc. of the liquid were inserted into these holes and sealed. One burette contained a portion of the boiled filtrate just referred to, and the other unboiled filtrate. In each case the solution was taken up by the tree in a few hours. On November 5, 1912, a considerable quantity of gum was observed pushing up into the burette which had contained the unboiled filtrate. No gum was observed in the other burette. No further development had taken place in either case on November 30, 1912.

EXPERIMENT 2.—On April 16, 1913, filtrate from a large piece of lemon bark containing a lesion 35 by 8 cm. produced by inoculation with *Pythiacystis citrophthora* (Pl. 3, B) was obtained as described in the previous experiment. At the same time, a filtrate from a large piece of sound bark from a healthy tree was obtained in the same manner, as a control. The filtrate from the diseased bark was placed in a separatory funnel and was allowed to be absorbed by a young lemon tree (Pl. 8) through a small hole in the bark. About 100 cc. were taken up in five days. The filtrate from the sound bark was placed so as to be taken up by another lemon tree of the same size.

On May 6, 1913, thin gum was observed pushing up into the lower end of the funnel tube through which diseased filtrate had been introduced. Gum undoubtedly had begun to form internally long before this time. In about two weeks the gum (about 10 cc.) had accumulated in the lower enlarged portion to a height shown in Plate 8, after which the pressure resulted in this breaking through the rubber connections at the bottom. Very little gum was exuded after June 3, 1913, when the funnel was removed. The bark remained alive around the opening. During this entire time no gum appeared in the funnel through which the filtrate obtained from the sound bark had been introduced.

The results of these experiments indicate that there is a substance in the diseased bark which is capable of passing through a fine clay filter and of inducing gum formation. In the experiments, however, gum formation soon ceased without serious injury to the bark, such as is produced by the invasion of *Pythiacystis citrophthora*. It would seem, therefore, that the gumming is induced by some substance that is formed either by the fungus or by the interaction of the host and parasite. The fungus during its invasion of the host probably secretes a substance which stimulates either directly or indirectly the production of a gum-inducing enzym by the host cells themselves. This substance doubtless also passes out into adjacent cells, and into the conducting tissue, and thus brings about gum formation at long distances from the invaded zone. This substance passing out from the invaded regions probably accounts for the large outer noninvaded gummous zones which so readily recover when the inciting cause, the parasitic fungus, is removed. This substance, capable of inducing gum formation, but not capable of bringing about the formation of lesions characteristic of Pythiacystis gummosis, appears to be destroyed by boiling and may, therefore, be an enzym. Further investigation as to the real nature of this gum-inducing substance is needed.

PARASITIC ORGANISMS AND GUM FORMATION

Although there are various agencies or diverse stimuli that contribute directly or indirectly to gum formation in Citrus, the more serious and progressive types of gummosis (in California at least) appear to be initiated by the invasion of certain parasitic fungi. With Pythiacystis gummosis, for sample, the bark and cambium region are first invaded and killed by the fungus which appears to advance most rapidly in the inner bark adjacent to the cambium. Soon after a small portion is killed by the initial invasion of the fungus, an influence is exerted bringing about gum formation in the xylem region surrounding the dead portion. After some time gum begins to form rapidly within the xylem for long distances vertically, but usually for only short distances laterally, from the invaded zone. The parasite, therefore, must stimulate such gum formation in an indirect manner, through setting into action some substance which passes out into the conducting system, and which moves probably in the sieve tubes as well as in the wood vessels. This gummy degeneration within the outer gummous zone usually does not kill the bark nor prevent its recovery, provided the parasite does not invade it later. A thin outer layer of wood, not penetrated nor killed deeply by the parasite, becomes infiltrated with gum, and occasionally gum pockets are formed within it but usually near the cambium.

The seriousness of excessive gum formation in itself has been overemphasized by some investigators because it was erroneously held that the death of the bark was the usual cause of gum formation instead of being the result of fungus invasion. Gum formation appears to diminish rather than to increase the seriousness of the diseases. As has been previously mentioned, the excessive gum formation in connection with Pythiacystis gummosis is frequently accompanied, or is soon followed, by a stoppage in the further advance of the invading parasite. It is highly probable that the infiltration of tissue by gum in the outer gummous zone tends to hinder the further advance of the mycelium into this tissue. In Diplodia gumming, Earle and Rogers (*21*) state that their observations have convinced them that gum flow in Citrus serves as a "protective device," and in plum wilt, Higgins (*39*) concluded that the gum served to hinder the advance of the organism by being formed in its path of advance. In the disease due to *Sclerotinia libertiana* on Citrus twigs, C. O. Smith (*54*) states that with the appearance of the gumming of the twig the further enlargement of the lesion is checked.

The gum in connection with Pythiacystis gummosis lesions, in addition to being a probable hindrance to the organism, serves in dry weather as a seal or covering to the wood beneath the killed bark, thus preventing the entrance of wood-destroying organisms and preventing excessive drying-out of the exposed wood. The gum being soluble in water, however, it is of little value for this purpose after the rains appear.

BRIEF GENERALIZATION ON GUM FORMATION

The explanation of gum formation itself, which seems to fit in best with the known facts and results obtained with Citrus, is that a substance of an enzymatic nature may be brought into action through various instrumentalities, such as substances acting under the influence of invading organisms, introduced or induced chemical stimuli, etc. It appears to be evident that the enzym immediately responsible for gum formation is

produced by the host tissue itself; otherwise it would be difficult to account for the gum formation induced by such a variety of chemical substances and agencies. In Pythiacystis and Botrytis gummosis, for example, there would seem to be two possible explanations of the manner in which the gum formation is brought about: (1) A substance secreted directly by the fungus sets in motion the gum-forming enzym or (2) a substance resulting from the interaction of parasite and host furnishes the means by which the gum-forming enzym is brought into action.

In case of the action of chemicals, such as corrosive sublimate and sulphuric acid, the substance might act also in either of two ways analogous to that given for the parasite, either directly in the same way as a secreted substance, or indirectly through a substance produced by the interaction of the plant tissues and the introduced chemical. The experiments with sterile filtrate from diseased and healthy bark previously described, taken in conjunction with the work of Floyd, Higgins, and others, appear to show that whatever may be the indirect factors in gum formation, the immediate cause or stimulus to its formation is a filterable substance of the nature of a heat-sensitive enzym. It is evident that many important phases of the subject of gum formation in Citrus remain for further investigation.

SUMMARY

(1) A destructive form of Citrus gummosis attracted attention first in the Azores in 1834. A similar gum disease appeared in Italy as early as 1863; in Portugal, 1865; in Australia, 1867; in Spain, 1871; in United States of America, 1875; and in most other Citrus regions before the year 1890.

(2) Pythiacystis gummosis, the most widespread and destructive type of Citrus gummosis in California, is characterized by copious exudations of gum and dead patches of bark on the trunk and main roots. The gum may arise not only from the margin of the invaded area but also from a large contiguous, outer, noninvaded zone.

(3) It has been shown that the disease with all its usual symptoms may be readily transmitted to healthy trees by inoculation with bits of bark tissue cut from the advancing margins of killed regions of bark. It is not transmitted, however, by tissue from surrounding outer gummous zones or by killed tissue not recently invaded.

(4) Cultural tests have shown that live mycelium of *Pythiacystis citrophthora* Sm. and Sm., formerly known as the cause for lemon brownrot, is present in this narrow band or fringe at the advancing edges of the killed region of bark (invaded zone) but is absent or dead elsewhere.

(5) Numerous inoculations with pure cultures of *Pythiacystis citrophthora* into healthy trees under various conditions have shown that this fungus is capable of inducing gummosis with all the characteristic symptoms of naturally occurring cases. The fungus has been reisolated from many lesions after it has remained in the bark from 1 to 11 months.

(6) Lemon fruits affected by brownrot due to *Pythiacystis citrophthora* have been shown to be capable of inducing the same type of gummosis as that produced by the fungus from gummosis lesions.

(7) The organism appeared to die out more readily and the lesions became self-limited more quickly after inoculation in branches or large roots than after inoculation in the trunk.

(8) A limited number of inoculation tests gave some indication that *Fusarium* sp., found commonly in connection with Pythiacystis gum-

mosis lesions, increased the severity of the disease when associated with *Pythiacystis citrophthora* but that Fusarium alone was not capable of initiating this gummosis.

(9) Observations and inoculation experiments both indicated that the order of resistance of species and varieties to *Pythiacystis citrophthora* from highest to lowest, is, sour orange (*Citrus aurantium* Linn.), trifoliate orange (*Poncirus trifoliata* Raf.), rough lemon (a resistant variety of *Citrus limonia* Osbeck), pomelo (*Citrus grandis* Osbeck), sweet orange (*Citrus sinensis* Osbeck), and common lemon (*Citrus limonia* Osbeck). The first is almost immune, the last very susceptible.

(10) Inoculation of small roots of young trees indicated that common-lemon roots are somewhat susceptible but that sweet-orange, pomelo, and sour-orange roots are resistant.

(11) Mal di gomma due to *Phytophthora terrestria* Sherb. has been shown to be similar to certain phases of Pythiacystis gummosis, especially to the form the latter takes at the junction of the main roots and trunk of old orange trees in California.

(12) Inoculations with *Phytophthora terrestria* and with *Pythiacystis citrophthora*, under the same conditions, produced lesions which showed no characteristic differences.

(13) Experiments have shown that in cases where excess of moisture and other contributing conditions cannot be entirely avoided the disease may be prevented largely by applying Bordeaux mixture or other fungicides to the trunks.

(14) It has also been shown that the progress of the disease may be readily prevented by dissecting out the bark invaded by the causal organism and applying a fungicide. It was not found necessary to remove the bark in the outer gummous zone, since this bark would finally recover after the advancing fungus had been removed.

(15) Botrytis gummosis of lemon trees is characterized in the early stages by a soft area of invaded bark killed to the wood, with exudation of gum on the trunk. Later this soft area becomes surrounded by a larger, firmer area in which only the outer layer of bark is killed, leaving a layer next to the cambium region alive. There is also, as in Pythiacystis gummosis, a noninfected outer gummous zone from which copious gum exudation may take place.

(16) A strain of *Botrytis cinerea* Lk. has been found commonly associated with this type of gummosis and has been isolated from numerous lesions.

(17) Pure cultures of the fungus, as well as bits of the diseased bark, were found capable of inducing the disease when inoculated into cuts and other kinds of injuries on healthy lemon trees.

(18) Attempts made to induce gum formation by various kinds of wounds on lemon tree trunks invariably failed when these wounds were kept clean and free from contamination with injurious organisms or chemical substances.

(19) The experimental results show that the disease may be prevented by avoiding injuries to the bark and by using a fungicidal coating on its surface. The treatment found effective consists in cutting or scraping away the dead bark, leaving as much of the live bark as possible, and painting the treated area with a fungicide.

(20) *Sclerotinia libertiana* is occasionally found associated with rapid dying of bark and copious gum exudation on trunks and roots. The

bark is at first soft, later dries out into long shreds, and usually contains flat sclerotia within it.

(21) Inoculations with pure cultures have shown that this fungus may also kill the bark rapidly and bring about the results just mentioned on healthy lemon tree trunks.

(22) Inoculation experiments with a large number of other organisms showed that some of them were capable of inducing gum formation, but this effect was usually slight as compared to that of Pythiacystis, Botrytis, and Sclerotinia. Those which produced some gum exudation in cuts were *Penicillium roseum*, *Fusarium* sp., *Diplodia* sp., *Coryneum berynkii*, *Coprinus atramintarius*, *Alternaria* sp., and *Hypholoma* sp. The effect in killing of bark was insignificant, and no definite diseases resulted. Several other organisms produced no effect whatsoever either in inducing gum exudation or killing tissue.

(23) In Parts I and II of this paper the relation of certain fungi to definite types of diseases in which gum is a conspicuous feature has been discussed.

(24) Gum in Citrus is similar to gum arabic and cherry gum and appears to originate mainly in the xylem tissue by hydrolysis of the cellulose walls.

25) Attempts to induce gum formation in lemon trees by various mechanical injuries, by obstructions placed in the conducting tissue, and by pressure on the bark, failed when the tissues experimented upon were free from parasitic organisms or unusual chemical stimuli.

(26) Injuries from certain insects have been observed to result in slight gum formation, which is probably due to secretions by the insects or to contaminating organisms.

(27) Experiments and observations indicate that burning and freezing are not important factors in inducing gum formation in Citrus but merely serve to open up the way for parasitic and wood-rotting fungi, which afterwards induce gumming.

(28) Partial desiccation appears to be merely a factor in the acceleration of the process of gum formation in Citrus and not a necessary condition to its initation.

(29) Certain chemical substances are capable of inducing gum formation when injected into Citrus bark. It has not been possible, however, to reproduce all the typical symptoms of any of the gum diseases by chemical injections. The invasion of certain parasitic organisms appears to be the chief factor in initating gum formation under natural conditions. These organisms may bring about, as do certain chemical substances, gum formation over considerable areas of bark surrounding the portions killed by either the organism or the chemical substance.

(30) Comparative experiments with boiled and unboiled filtrates from diseased and healthy tissue show that the diseased bark contains a substance capable of passing through a fine clay filter and of inducing gum formation when unboiled. When boiled, however, this capacity to stimulate gum is destroyed. This indicates the presence of a heat-sensitive enzym.

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PLATE 1

Pythiacystis gummosis on lemon trees, from inoculation with diseased bark tissue.

A.—Tree inoculated on February 27, 1912, the gum formation showing the development of disease on August 2, 1912.[a]

B.—Same tree, September 19, 1912, over 6 months after inoculation.

C.—Same tree about 1 year later, view at right angles to B. Only 5 cm. of live bark then prevented complete girdling.

D.—Same view as A and B, on May 24, 1913, 15 months after inoculation. The gum first formed has been dissolved away by winter rains and the dead bark has dried and shrunk. Gum was exuding farther around, as in C, at this time.

E.—Lemon tree inoculated with diseased bark tissue, September 21, 1912, showing excessive exudation of gum 2 months later.

F.—Lemon tree inoculated with diseased bark under a glass slide held with putty, September 21, 1912, showing gum formation 2 months later.

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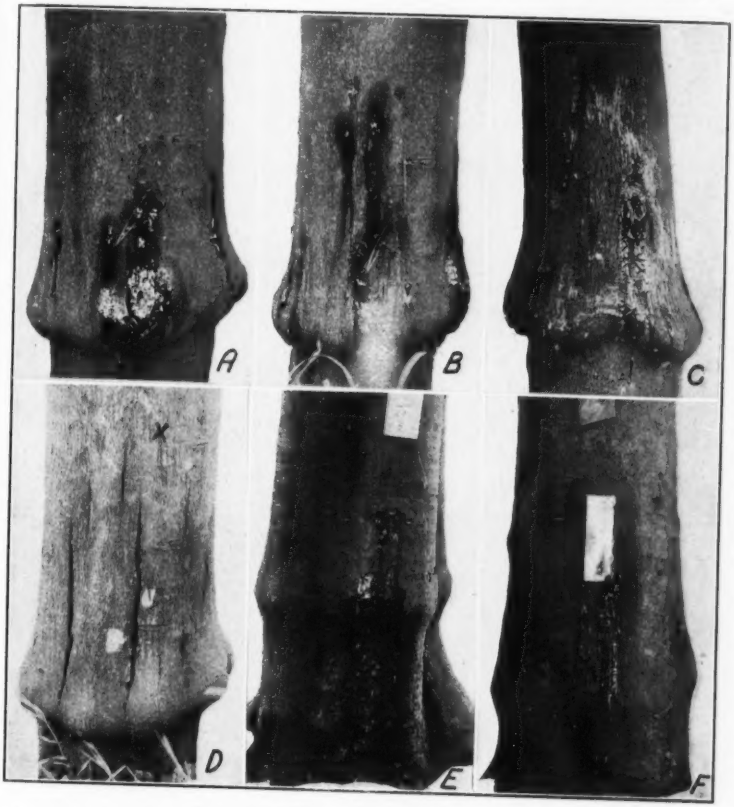
[a] A majority of the trees in this same orchard had the same "overgrowths" at the union of stock and scion as observed in this illustration.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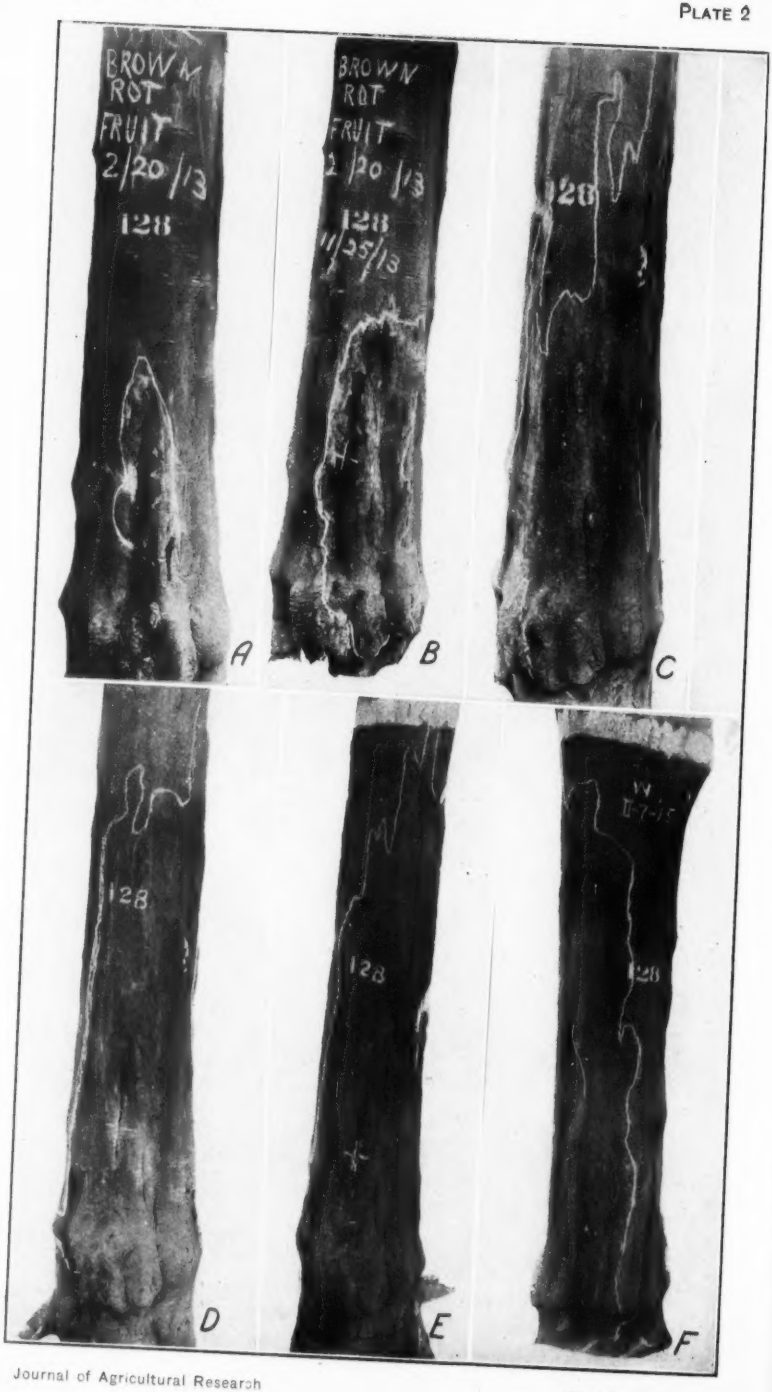
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PLATE 2

Pythiacystis gummosis on lemon tree from inoculation made on February 20, 1913, with diseased fruit tissue affected with Pythiacystis-rot.

A.—Extent of invaded bark and gum formation about 5 months after inoculation.

B.—Same tree about 7 months after inoculation, showing increase in the invaded area.

C.—Same tree about 13 months after inoculation, showing cracking of dead bark over invaded area and its large increase in size.

D.—Same tree 18 months after inoculation, showing a still larger area affected.

E, F.—Two views of the same tree, about 2 years after inoculation. Figure F taken on opposite side of tree from figure E.

PLATE 3

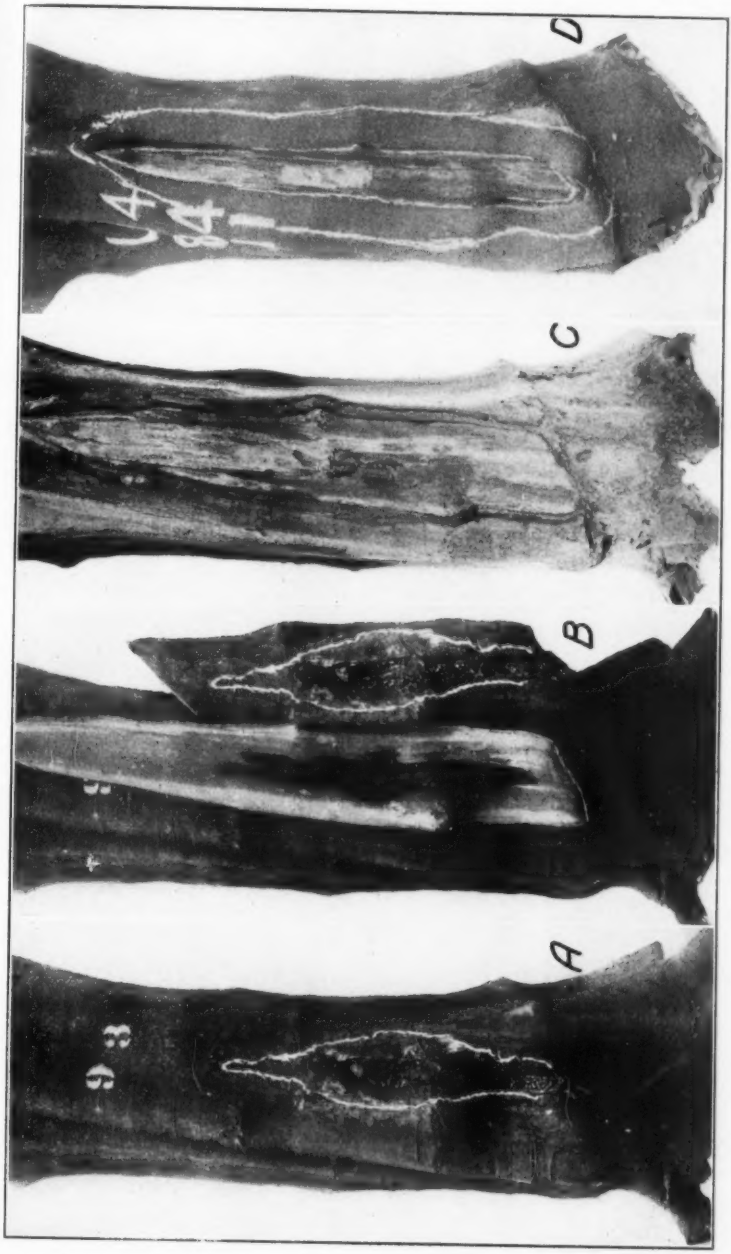
Pythiacystis gummosis on lemon tree from inoculation on November 23, 1912, with pure culture of *Pythiacystis citrophthora*.

A.—Extent of invaded portion (inside of chalk line) and gum formation on June 6, 1913. The fungus was isolated from several places near chalk line at this date.

B.—Same tree after bark was cut, showing invaded zone (black) and a part of the outer gummous zone (shaded), which extended upward and downward under live bark.

C.—Same tree in September, 1914, showing new bark pushing in over wound.

D.—Same tree in June, 1920, showing increase in the new bark covering the edges of the original wound.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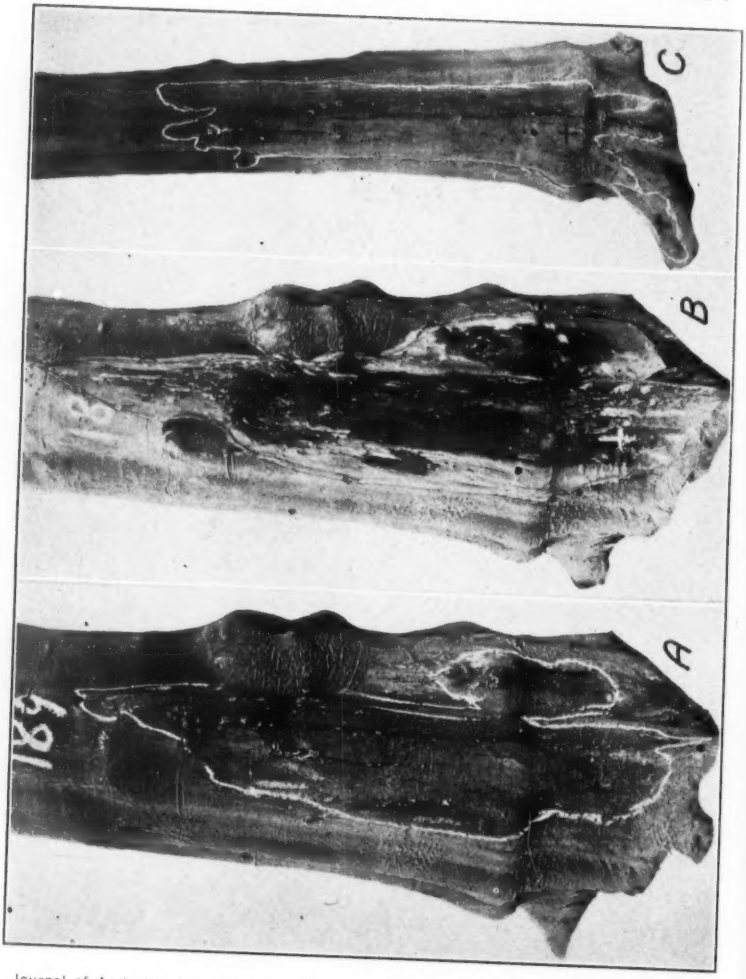
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PLATE 4

Pythiacystis gummosis from inoculations made July 13, 1913, with pure cultures of *Pythiacystis citrophthora* on different parts of the same lemon tree.

A.—Extent of invaded zone and gumming from an inoculation on the sweet-orange stock. Photographed May 9, 1914. The fungus was isolated from upper portions near chalk line at this date, about 10 months after inoculation.

B.—Same, with bark removed, showing darkening at cambium region over invaded zone. Original inoculation was at the place marked +, from which the invasion of the fungus proceeded mostly upward, over the "bud union" (swollen ring) into the lemon bark.

C.—Opposite side of same tree on same date, showing portions invaded (chalk line) as result of two inoculations, one on lemon bark above and one on a large crown root below the bud union.

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PLATE 5

Pythiacystis gummosis which was apparently increased in severity by inoculation with both *Fusarium* sp. and *Pythiacystis citrophthora* February 22, 1913, showing progress of disease over a period of about 3 years. This tree was finally girdled and killed by disease starting from one inoculated cut.

A.—Exuded gum and extent of invaded zone (chalk line) July 28, 1913, 5 months after inoculation. Arrow indicates point of inoculation.

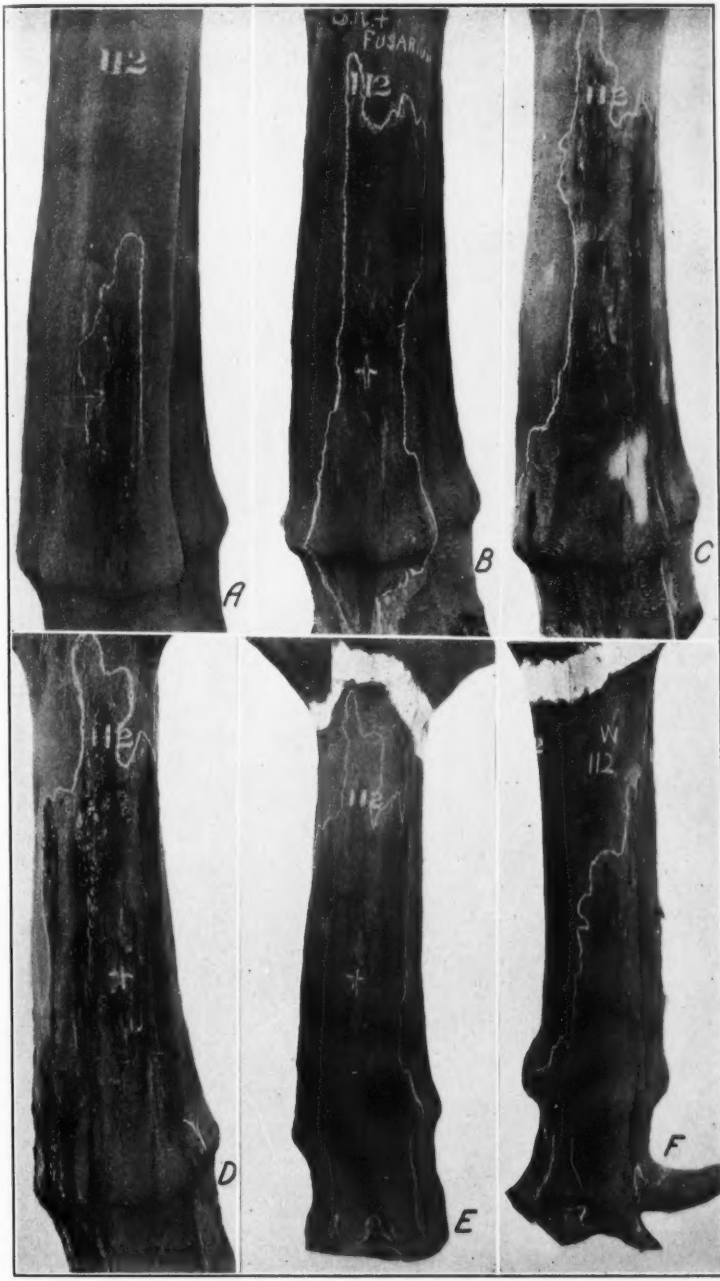
B.—Extent of the lesion on same tree November 25, 1913, 9 months after inoculation. The infection has extended downward nearly as far as upward.

C.—Extent of lesion and cracking of bark on April 9, 1914, more than 13 months after inoculation. The gum of figures A and B has been dissolved by rains.

D.—Large quantity of exuded gum and a great enlargement of invaded zone and cracking of bark, on September 4, 1914, about 17 months after inoculation.

E.—Further cracking of dead bark on February 6, 1915, nearly 2 years after inoculation. The gum of figure D has been dissolved by further rains.

F.—Trunk on opposite side from figure E, on same date. Only a small strip of live bark here remains, which was later invaded, this resulting in the death of the tree.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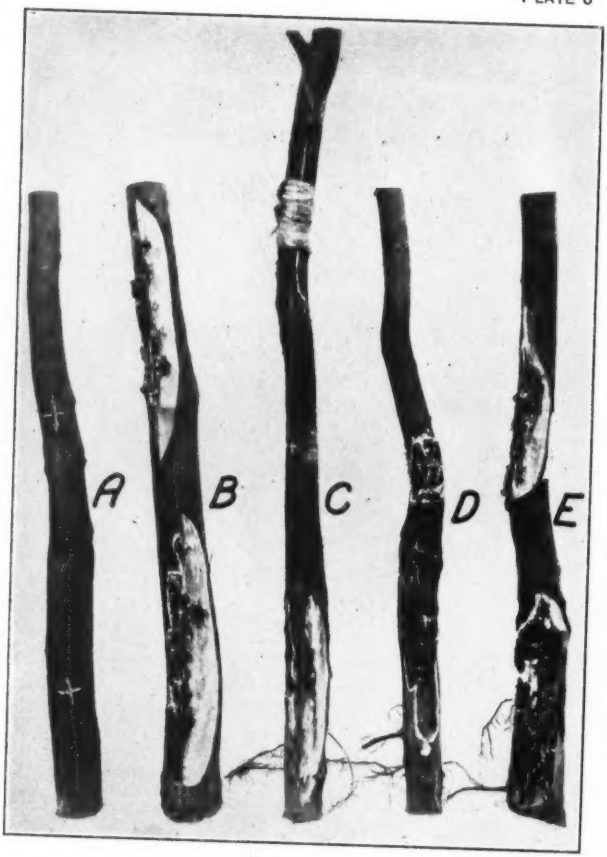
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PLATE 6

Results of inoculation with pure cultures of different strains of *Phytophthora terrestria* and with *Pythiacystis citrophthora* on young lemon trees (orange stock at lower inoculated cut). Inoculated April 20, 1914, photographed August 26, 1914. (See Table XI.)

A.—Control cuts without inoculum.

B.—Inoculated with *Phytophthora terrestria* from Isle of Pines. Bark cut away to show extent of lesions.

C.—Inoculated with *Pythiacystis citrophthora* from California.

D.—Inoculated with *Phytophthora terrestria* from Cuba.

E.—Inoculated with *Phytophthora terrestria* from Florida.

PLATE 7

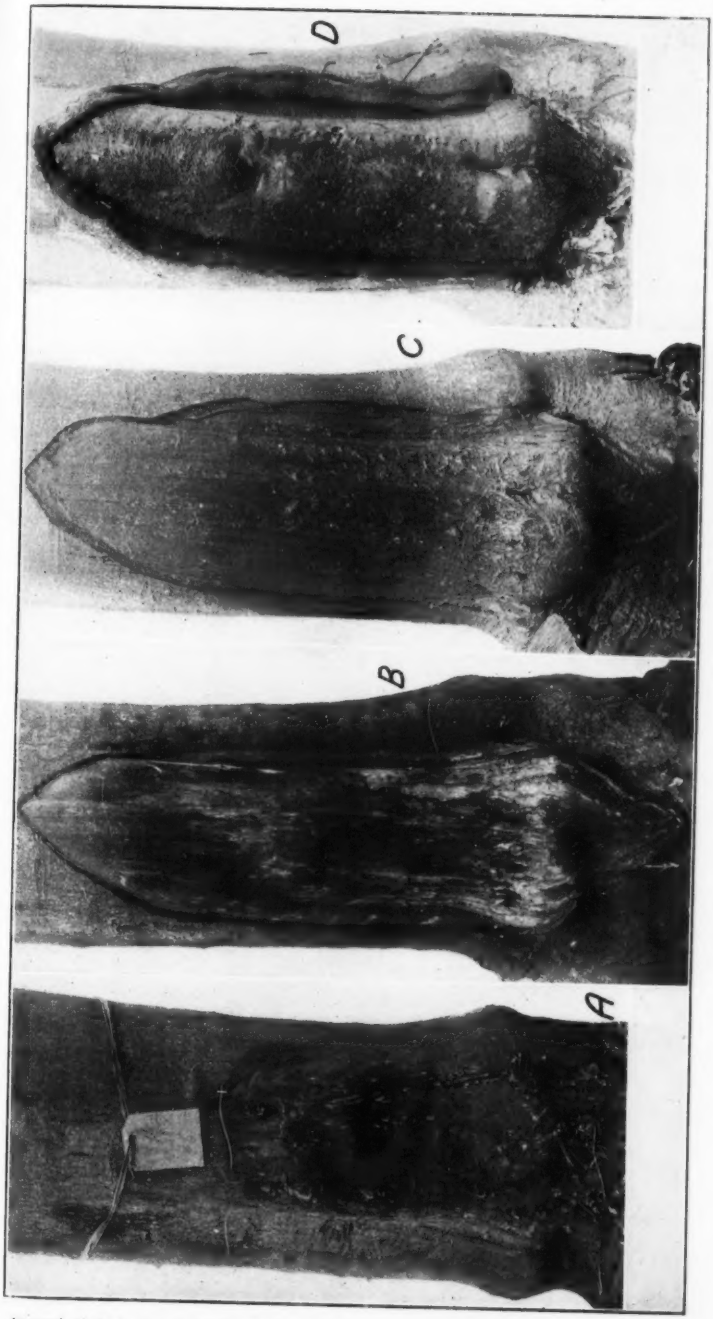
Method of cutting away diseased bark in treatment of a severe case of Pythiacystis gummosis.

A.—Result of inoculation with diseased bark containing *Pythiacystis citrophthora* on November 16, 1912. Photographed September 3, 1913. Invaded area 21.5 by 7.5 cm.

B.—Bark cut away September 3, 1913, on a similar tree in treatment.

C.—Same tree after painting with Bordeaux paste.

D.—Same tree on March 17, 1916, showing growth at edges of wound and asphalt paint on exposed wood.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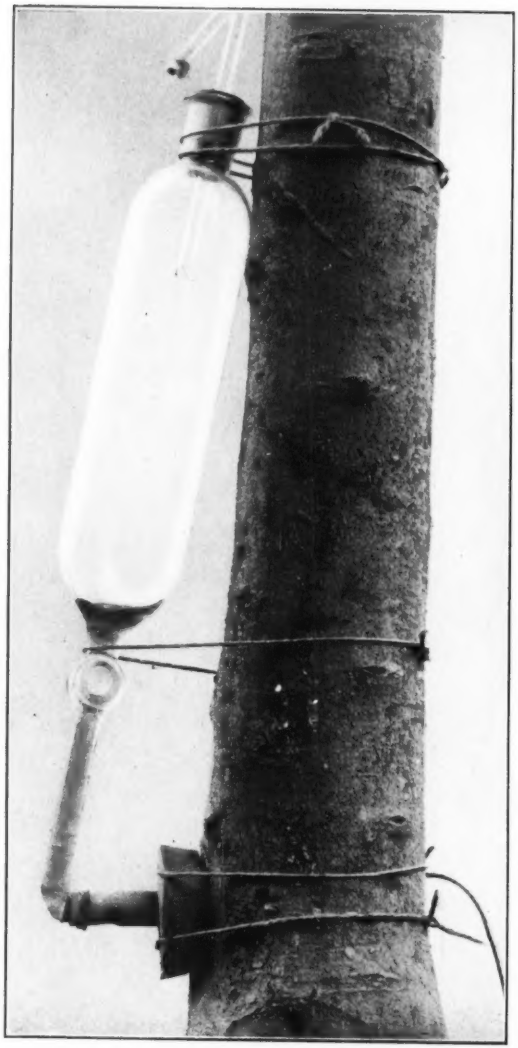

PLATE 8

Gum pushed up into bottom of funnel from which sterile, unheated, and filtered extract from bark affected with Pythiacystis gummosis had gone into the tree about 4 weeks before.

OCCURRENCE AND SIGNIFICANCE OF PHLOEM NECROSIS IN THE IRISH POTATO[1]

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INTRODUCTION

In a recent publication Esmarch (*5*)[2] makes the following generalization in regard to the condition of the phloem:

> Necrosis of the phloem is found in both normal and diseased plants and is always present in mature organs; if necrosis is observed during the early life of the plant, it is an indication of premature ripening.

In support of this theory, which is based on his own observations and supplemented by the data of other writers (*7*), Esmarch compares the changes in the phloem of the potato to those taking place in the secondary phloem of woody plants. Here, with the disintegration of the nucleus and the formation of callus deposits over the plates, the sieve tubes become inactive. The empty elements, deprived of their turgor, collapse and are crushed by the surrounding tissues. As obliteration progresses the old phloem often becomes so changed structurally and chemically as no longer to resemble its former state. However, while the obliteration of the phloem in woody plants, as has been shown by numerous investigators (*3*), is comprehensible on structural and physiological grounds, similar changes in the phloem tissue of herbaceous plants may not be expected, nor is their frequent occurrence reported in literature. Indeed, our present knowledge is restricted to two short notes by Boodle and Schuman. Boodle (*4*) observed in *Helianthus annuus* the occurrence of sieve tubes and companion cells whose walls were lignified and whose content gave reactions resembling those of lignin. Schuman (*8*) mentions that sclerosis of the phloem takes place in some few woody Composites and gives *Scorsonera hispanica* and *Aster thyrsiflorus* as examples. A systematic study of the mature phloem, carried on recently in the botanical laboratory of Cornell University, indicates clearly that most herbaceous plants do not exhibit any changes in the phloem upon maturation; where changes do take place, the phloem elements become lignified without obliteration.

A consideration of anatomical changes in diseased plants has often been fruitful in providing diagnostic symptoms for the identification of plant diseases, provided, however, that the ontogeny of the normal parts was fully understood. It is imperative, indeed, to distinguish clearly between normally expected tissue changes, such as take place

[1] Accepted for publication July 11, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 245.

in the phloem of woody plants, and induced abnormal states, the latter alone being truly pathological. The apparent discrepancies noted by different investigators may, and usually do, become intelligible if we consider all pathological changes and trace the causes of the various types of necrotic conditions, if possible, to their sources.

A classical example of such discrepancies is afforded by the researches on the pathological anatomy of the potato in connection with leafroll. To be sure, the views of the different writers are substantiated by observations, but since statistical data are incomplete at best, only a purposeful and systematic investigation of the problem can provide adequate data, even though factors which govern plant growth be not altogether neglected in the analysis of results.

To arrive at some definite basis as to what constitutes a healthy potato plant from the viewpoint of the anatomist, and under what conditions the phloem will remain normal, an investigation of a large number of plants of both cultivated varieties and indigenous South American forms was carried on. Such studies have been in progress since the summer of 1916, and the data obtained have been embodied in part in earlier publications (*1*, *2*) and have been extended and partly modified in the present paper. Further work on the pathological anatomy of potato diseases will doubtless bring forth new interesting facts and greatly aid in analyzing these complicated potato disorders of which the cause is only vaguely understood.

THE NORMAL PHLOEM

The vascular tissue of the potato plant shows a bicollateral arrangement of its elements, a condition most clearly seen in the larger stem bundles (fig. 1). The primary phloem, external to the cambium, is made up of small groups of cells more or less continuous; the groups constituting the inner phloem are very variable in size and scattered. Groups of primary phloem appear also in the interfascicular region, where they may be seen on both sides of a well-developed cambium. Through branching and anastomoses the individual groups in each region communicate with one another, while through branch and leaf gaps a similar connection is effected between the inner and outer phloem.

During the early vegetative development only groups of primary phloem are seen in a cross-sectional area of the stem. However, when tuber formation is under way, the cambium gives rise to a broad band of phloem (fig. 2). The amount of this secondary phloem varies with the location. In the nodal region the amount exceeds that found in the internode, and in a given cross-sectional area, the larger amount is always found on the face of the larger stem bundles.

While secondary phloem elements become differentiated and take part in the translocation processes, the primary phloem groups remain active until the plant is mature. The walls of the cells thicken slightly, and occasionally callus deposits cover the plates of the sieve tubes. Otherwise, there are no noticeable changes, either structural or chemical, characteristic of the phloem of the mature plant.

Local necrotic changes in the parenchymatous tissue, however, may be observed in any potato plant. The diseased areas are usually restricted and do not extend vertically for any appreciable distance. In the distal stem region, especially the node, such small pathological areas are of frequent occurrence, but they usually disappear in the maturing

organs. The first appearance of such a diseased area is exhibited by a swelling in the cell wall, often accompanied by discoloration. At a later stage, browning of the walls is noted, while at the same time the

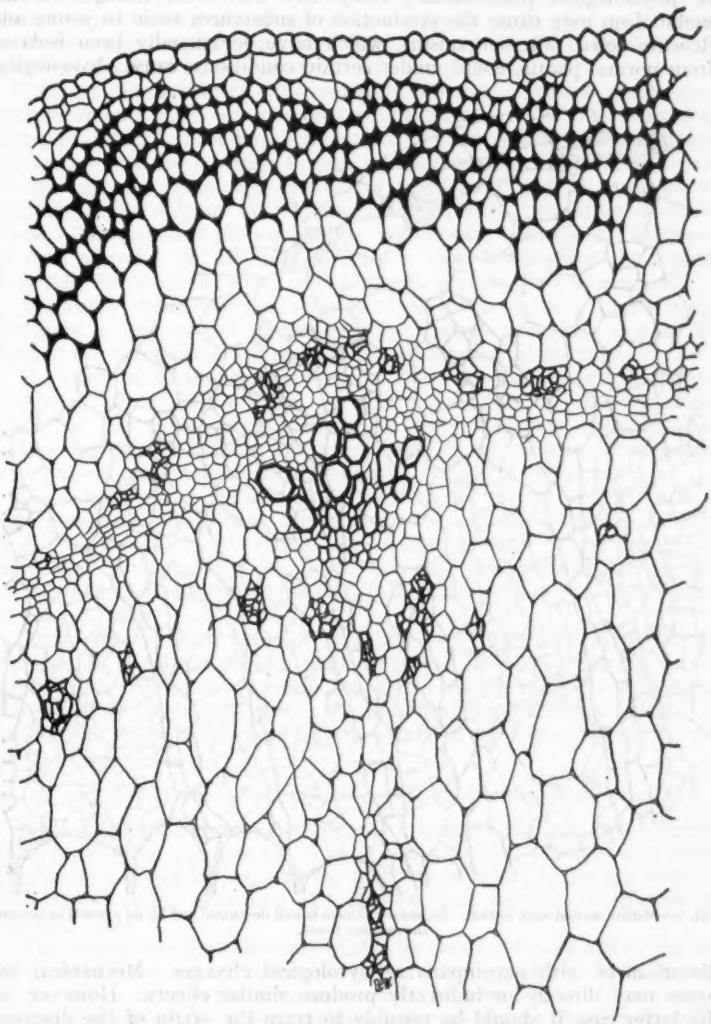

FIG. 1.—Stem section of young normal potato plant, showing general anatomical arrangement of vascular and fundamental tissues.

lumen is partly or entirely filled with a gummy deposit which is homogeneous or granular in nature. The parenchyma cells in the region where phloem fibers are differentiating show commonly an abnormal swelling, local in nature or extending laterally to include the walls of the perihperal phloem cells.

Any theory which could be postulated to explain the pathological disturbances in seemingly healthy individuals would naturally contain much of the hypothetical and be antecedent to our present knowledge of physiological phenomena. Temporary and local changes in the metabolism may cause the production of substances toxic to young and delicate cells. Microorganisms, which have occasionally been isolated from normal plants, could, under certain conditions, cause physiological

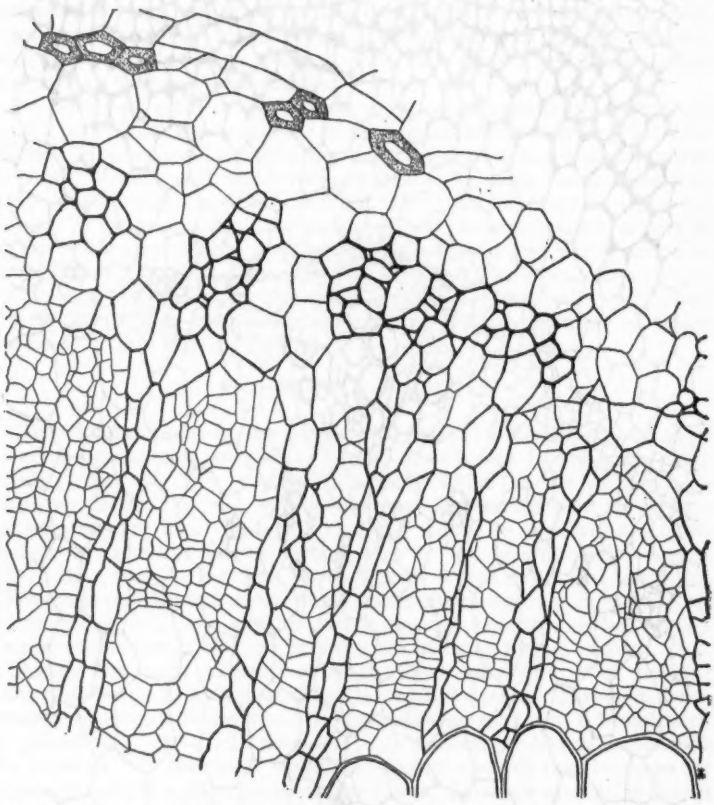

FIG. 2.—Mature normal stem section. Secondary phloem is well developed and by far exceeds in amount the primary tissue.

disturbances with accompanying cytological changes. Mechanical injuries may directly or indirectly produce similar effects. However, in the latter case it should be possible to trace the origin of the diseased area and to determine whether the cause was external or internal, and this has often been accomplished.

INFLUENCE OF ENVIRONMENT

An insight into the effect of environmental factors on the anatomical structure was obtained by studying a number of potato varieties grown at the high altitude station at Fort Lewis in Colorado. By taking ad-

vantage of the natural great diversity of ecological conditions, almost any desired combination of soil, moisture, and climatic factors could be had. Only where the natural agencies were inadequate or where a special combination of factors seemed advantageous was artificial control of these factors resorted to. Material for the study of the effect of progressively increasing water supply was taken from a field a part of which had become inundated by seepage from an adjoining mesa.

Plants suffering from an oversupply of water showed anatomical changes resembling those observed in foot diseases. The primary xylem and parts of the secondary elements of the wood were discolored and the lumen was filled with a brown, gummy deposit. The phloem part of the vascular tissue was commonly unaffected, and in cases where necrosis of the phloem was observed the material came from doubtful stock. The diseased phloem groups, which were occasionally observed in such material, did not take the lignin stain but showed the typical brownish discoloration which is characteristic of necrosis in general.

A greatly reduced water supply, or alternate wetting and drying, produced a denser and more strongly lignified wood. It neither inhibited nor accelerated the production of pathological changes.

The effect of shading was found to be closely bound up with the photosynthetic activity of the plant. Plants which grew to maturity in the shade of the river bottom vegetation were rank and slender. The xylem was greatly reduced and lignification of the cells less pronounced. The phloem remained normal throughout the life of the plants.

Thus, qualitative changes in the phloem, as a result of extremes of environmental conditions, are uncommon. When present, the diseased groups are localized and are connected with or are under the influence of necrotic areas in adjacent tissues. The pathological changes in the phloem tissue consists in a browning, rarely a typical lignification of the cell walls.

These observations, together with the fact that obliteration of the phloem, as a natural phenomenon in normal herbaceous plants, is discredited on theoretical grounds and disproved by extensive systematic studies, emphasized once more the fact that generalizations based on inadequate observations may lead us astray in the interpretation of true pathological changes.

NECROSIS FOLLOWING EXTERNAL INJURIES

By far the most common source of internal stem lesions is external injury produced by insects. The ensuing cellular changes may either constitute a direct response to the mechanical injury or be the result of irritations causing abnormal cell division and necrotic changes in the affected areas.

The lesions become externally visible as abnormal swellings of the size of small pustules barely discernible to the naked eye (Pl. 1, A) or they are of the nature of large intumescences which may be seen in various stages of development. The oldest swellings are conspicuous by their brownish color and the fissured surface of the epidermis. The location of the lesions is the apical stem region and the young petiole. However, swellings may be found in any region regardless of the age and relative development of the organ. Concomitant with the formation of these intumescences is a change in the morphological development of the parts above the points of injury. The leaves are deformed and discolored. The leaflets fold along the midrib or roll in tubular fashion. The inter-

nodes are shortened and often appear almost telescoped. Withal, the morphological changes are of a type akin to the symptoms of leafroll and foot diseases.

An examination of a section through a swollen area discloses to the naked eye a dark discoloration of the tissues. A microscopic study shows further that both vascular and cortical tissues are affected and that aside from necrotic changes there are regions of abnormal cellular activity. Sections through the advancing margin of a young pustule show that the first evidence of pathological changes is found in the cambium region. The xylem has matured irregularly, giving the peripheral region of the tissue a jagged appearance. In places one may observe mature groups of xylem embedded in parenchymatous tissue and completely separated from the vascular ring. In the more central part of the swelling the tissues appear completely disorganized. In cortex and pith are small groups of brown cells surrounded by concentric rings of thin-walled periderm cells. This latter type of necrosis is, however, frequently met with in normal plants, or is found in connection with other injuries.

The phloem tissue, in the affected areas, shows the same pathological condition observed in general. The cell wall and content show a brownish discoloration, and in extreme cases entire cells or cell complexes may be obliterated.

NECROSIS IN CONNECTION WITH STEM STREAK

Potato tubers of the variety Irish Cobbler which were grown in the greenhouse developed extensive stem lesions on both young and maturing shoots. The lesions may best be referred to as "streak," the affected areas being elongated and brownish in color. Their advance along the stem is acropetal. In the nodal region the browning is especially pronounced and extends into the lower part of the petiole.

The lesions are the result of necrotic changes in the cells of the collenchyma and adjacent tissues. The hypodermis is often still green while the collenchyma cells underneath are brown and in a state of obliteration. Soon, however, being cut off from the water supply, both epidermis and hypodermis die and the walls take on the same brown color as the cells underneath. In advanced lesions the necrotic areas extend through cortex and vascular tissue into the pith. In the nodal region the entire pith is reduced to a cavity lined with obliterated and discolored parenchyma cells. If the epidermis from an advanced lesion is removed and cleared in chloral hydrate, the microscopic examination shows scattered through the homogeneous mass of brown cells small areas of dark-colored cells. These areas usually cluster around a stomate and extend centripetally into the pith.

The behavior of the phloem cells is of special interest in this connection. Severe necrosis may be observed in both inner and outer phloem (Pl. 2). The cells are brownish in color, the lumen distinct or partly closed by the pressure of the surrounding tissue. Characteristic intercellular spaces are also formed, and progressive lignification of the type diagnostic of leafroll is not uncommon. The necrotic phloem groups, however, seem to be restricted and limited by the extent of the external symptoms, there being no regularity either in their vertical or lateral distribution. It still remains to be shown whether this disease is definitely connected with leafroll, and, if in part distinct, whether certain aspects of the disease are not the result of secondary infection by the leafroll virus.

TRUE PHLOEM NECROSIS AND THE LEAFROLL PROBLEM

In the microscopic examination of potato vines one observes, occasionally, pathological changes associated with certain types of diseased plants which by virtue of their peculiar external appearance are grouped under the collective name of leafroll. Quanjer (*6*), while investigating the leafroll disease, noticed this correlation, and as the result of subsequent studies arrived at the following conclusion:

> The lignification of the phloem is a dependable diagnostic symptom for the identification of leafroll, and the physiological disturbance, occasioned by the destruction of the conducting system for plastic materials, accounts for the change in the morphological structure and for the reduction in yield.

Stem sections of a typical leafroll plant exhibit, as a diagnostic internal symptom, a necrosis and lignification of the phloem groups. In case of severe external symptoms the diseased groups pervade the entire plant with the occasional exception of the underground organs. The distal stem region is commonly affected, and in nearly every instance the necrotic changes are of an extreme type. The basal stem region always shows necrotic changes when external symptoms become evident while the plant is still young. As a rule necrosis of the phloem in the lower stem means general necrosis of the plant throughout its extent, but the symptoms may decrease toward the distal end or disappear altogether. At any given height of the stem the node is typically more severely affected than is the internode. This condition is especially observed in the initial stages of the disease, but during subsequent development either region may be equally affected.

In petiole and midrib necrotic phloem groups may appear at a much later period, but the extent of necrosis is as a rule correlated with the severity of the rolling and the discoloration of the foliage. In the young leaf most advanced necrosis is observed in the middle part of the petiole probably because this part of the leaf is ontogenetically the oldest. In mature leaves, however, the midrib is often the organ which is most severely affected.

In the underground organs of diseased plants the phloem strands are usually normal, but in severe cases both stolons and tubers will show evidence of necrosis.

The lateral distribution of phloem necrosis is also subject to a great deal of variation. This is true for the phloem groups of a certain region, but the difference becomes even more apparent when outer and inner phloem are compared. In a given cross sectional area one may observe phloem groups in various stages of degeneration. Entire groups may be affected in whole or in part. Often one notices perfectly healthy groups side by side with diseased ones, which seems the more remarkable if one recalls how closely the groups are connected with each other through branching and anastomoses. In the apical stem region the first stages of necrosis are found in the external phloem and only later in both regions. In the basal stem either region is found to be diseased, but often the inner phloem alone is completely destroyed while the outer phloem is altogether normal or shows, at the most, only initial stages of disorganization.

In petiole and midrib, necrosis is primarily restricted to the outer phloem. In advanced stages the bundles flanking the corners of the large lateral bundles are necrotic, and occasionally all the inner phloem is also diseased.

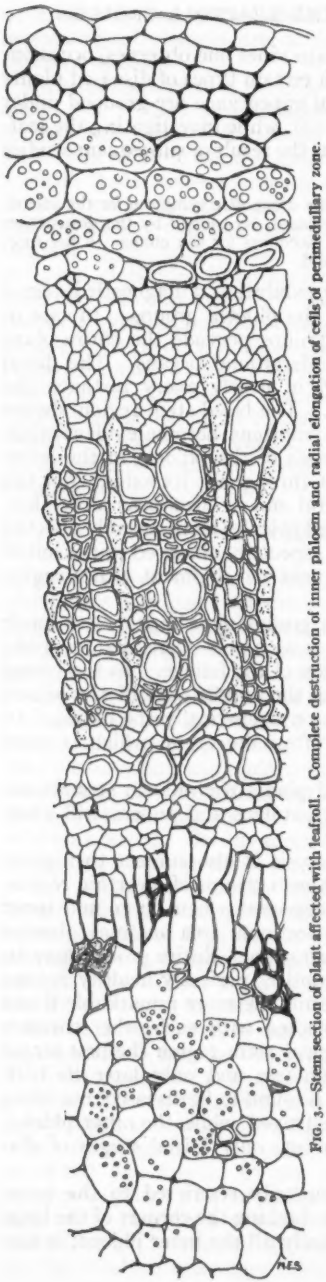

FIG. 3.—Stem section of plant affected with leafroll. Complete destruction of inner phloem and radial elongation of cells of perimedullary zone.

The progressive lignification of the phloem tissue can best be studied in sections taken through the distal stem region. Here one usually finds a complete series of changes from the first cell response to complete disorganization of the elements. Before there is any detectable evidence of lignification the development of the vascular tissue shows a deviation from its normal course, which is indicated by a more irregular maturing of the xylem than is found in healthy plants (Pl. 1, B). If such a section is stained with phloroglucin and hydrochloric acid, one notices upon close observation that the phloem cells centrifugal to the depression in the cambium show a slight amount of lignification in parts of the wall.

The cells of the pericycle in this region differ also from the normal type in having a greater radial diameter. As lignification progresses the entire cell wall or entire groups of cells become affected, while at the same time the radial elongation of the pericyclic cells of the outer phloem and the parenchyma of the perimedullary zone of the inner region becomes more pronounced (Pl. 3). The cells which first show lignification are commonly found adjacent to the fibers, but now and again lignification in a phloem group may start at the center and extend in a centrifugal direction.

Prior to lignification of the phloem, to be tested microchemically, a swelling of the walls of the diseased cells takes place. Ferrous sulphate and potassium ferrocyanid at this stage impart a deep blue color to the walls, indicating the presence of large quantities of pectic substances. The swelling of the walls extends from the region of the fibers centrifugally (Pl. 4, B). Gradually the cells lignify and progressively cells and groups of cells are withdrawn from active participation in conduction. In severe cases most or all of the primary phloem becomes destroyed (fig. 3; Pl. 5). In the initial stages of lignification of certain phloem groups the primary walls of adjacent cells swell and separate. The intercellular space

thus formed becomes filled with a brown deposit, which at a certain stage takes the lignin stain. Following the swelling of the wall there is often a disintegration of the wall substance. The swollen wall appears lamellate (Pl. 4, A) and between the lamellae spaces are formed which are filled with a gummy substance. The cell content also degenerates and disappears in part or becomes transformed into a substance giving reactions similar to the interstitial substance described above. Following the death of the cell and the subsequent loss of turgor, the phloem elements collapse, unless rapid lignification lends sufficient rigidity to the walls and prevents their being crushed by the surrounding tissues.

CONCLUSIONS

The phloem of the potato, like that of the majority of herbaceous plants, remains normal throughout the vegetative period and up to late maturity of the plant. Although it is not affected by extremes in environmental conditions, it nevertheless undergoes pathological changes under the effect of certain metabolic disturbances such as probably exist in the leafroll disease. These changes consist in lignification or obliteration of cell wall and content. However, while obliteration of the phloem is always observed in connection with leafroll, it is also an accompanying phenomenon in other diseases. It is not so much its mere presence as its universality in distribution, coupled with the absence of necrosis in other tissues, which gives it a real diagnostic value.

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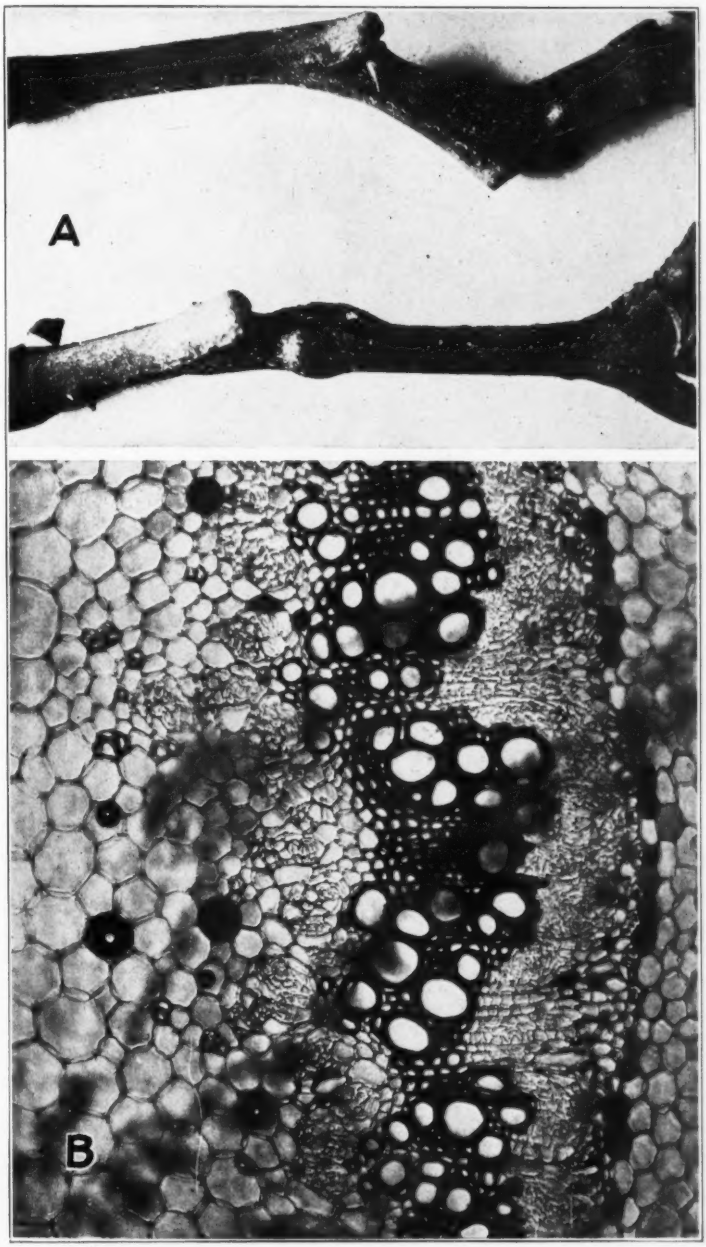
PLATE 1

A.—Distal stem region. The swellings are due to insect injuries; they show as internal symptoms necrosis of vascular and cortical tissue.

B.—Hand section of potato stem affected with leafroll. The xylem is maturing very irregularly, which gives the cambium zone a jagged appearance.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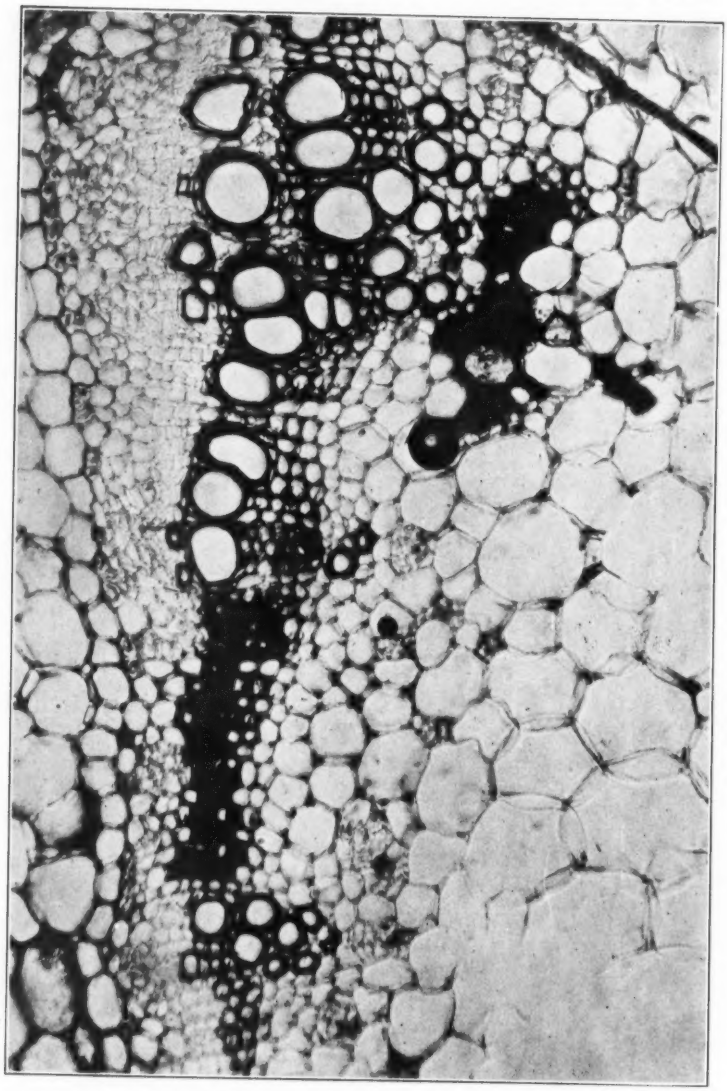

PLATE 2

Hand section of potato stem affected with "stemstreak." Both xylem and phloem show necrosis. The necrosis of the phloem, however, is not a typical lignification.

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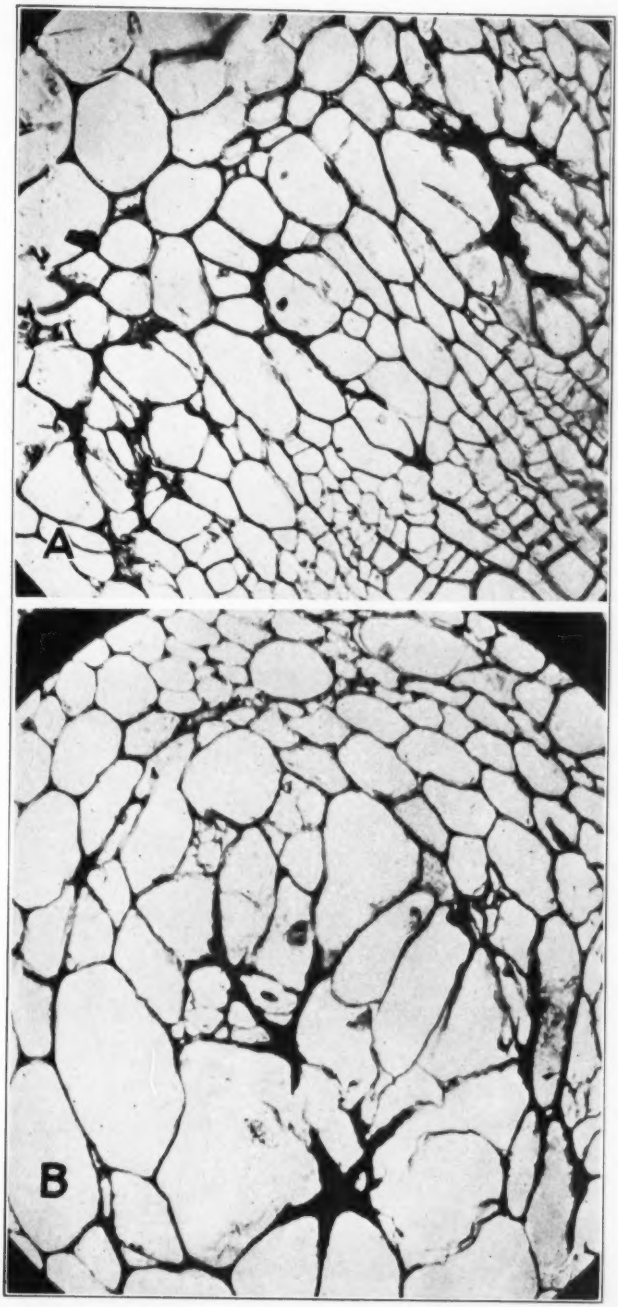
PLATE 3

A.—Advanced necrosis of outer phloem in leafroll stem.
B.—Advanced necrosis of inner phloem.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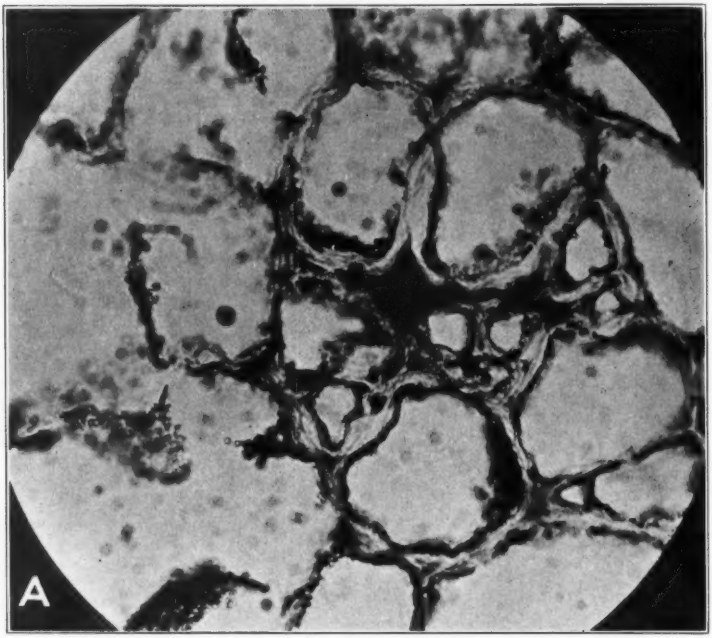
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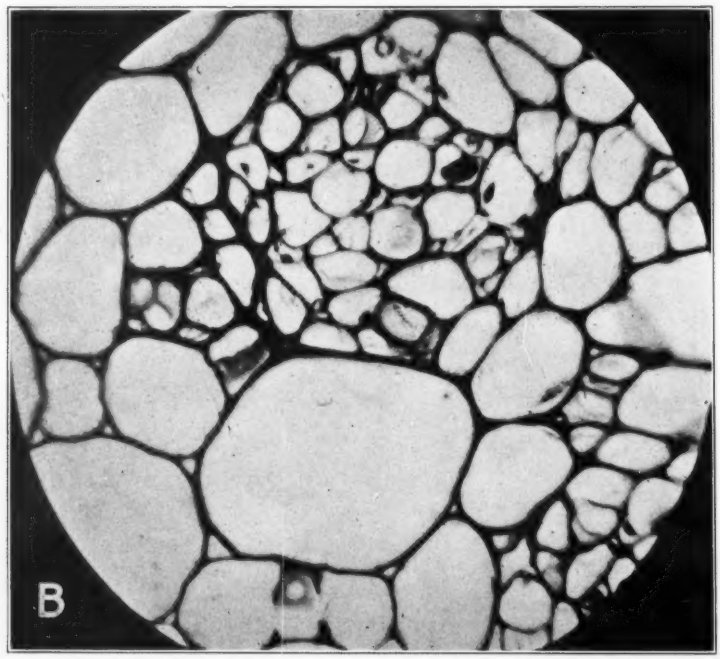
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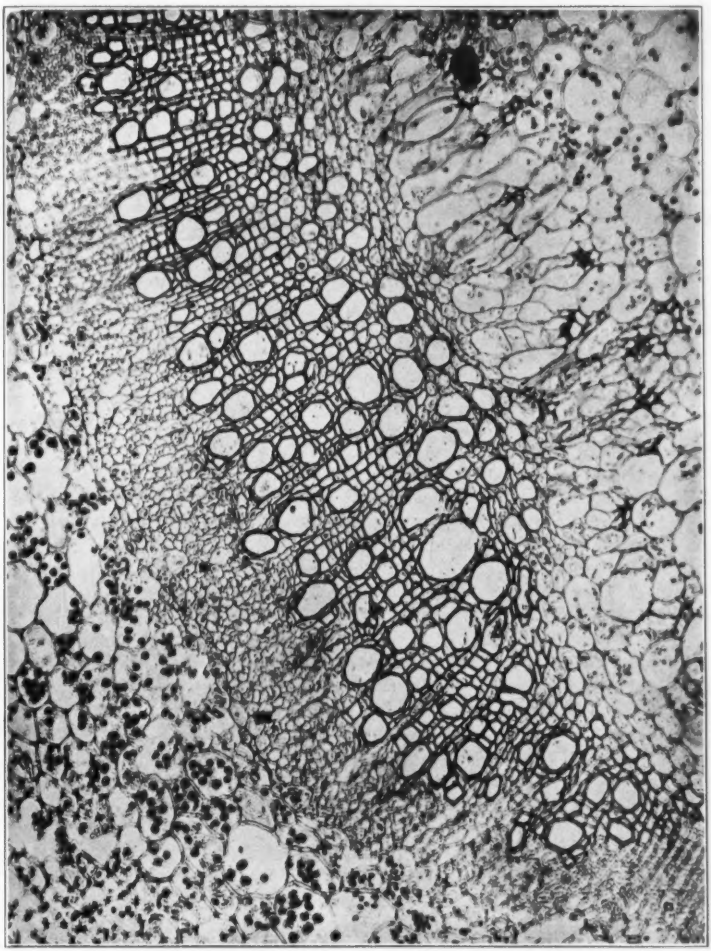
PLATE 4

A.—Enlarged view of inner phloem group, showing the minute pathological changes in the structure of the cell wall. (Gold chlorid stain.)

B.—Inner phloem group of potato stem, showing beginning stages of necrosis. Note the swelling of the walls of certain of the cells in the region of the fibers.

PLATE 5

Section through a large stem bundle of a mature stem affected with leafroll. The inner phloem is completely destroyed while the outer phloem, on the other hand, is normal.



CULTIVATED AND WILD HOSTS OF SUGAR-CANE OR GRASS MOSAIC[1]

By E. W. BRANDES, *Pathologist*, and PETER J. KLAPHAAK, *Assistant Pathologist*, *Office of Sugar-Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

PURPOSE OF THE INVESTIGATION

Since 1919, when it was demonstrated by the senior writer that grasses other than sugar cane are susceptible to the so-called sugar-cane mosaic (*3, 4, 5*),[2] a large number of experiments have been performed to determine whether grasses in general are susceptible. It was especially important to learn whether the forage and field crops of the South are affected and, if so, to what extent damage is caused. On account of the comparatively recent introduction of the disease on sugar cane into this country, it was expected that crops in the vicinity of diseased sugar cane would be more likely to be affected. Accordingly these crops were closely scrutinized for symptoms similar to those on sugar cane, and when suspected cases were observed the plants were tested experimentally at Washington. Other closely related cultivated plants were tested, whether or not they showed signs of natural infection in the fields.

Another class of grasses, namely, the weeds or wild grasses found in sugar-cane fields, was held under observation, and any species which showed signs of disease were similarly tested. The importance of having exact knowledge of the susceptibility of wild grasses to grass mosaic lies in the fact that the disease may be carried over winter in perennial grasses and may reinfect the susceptible annual grass crops in the spring.

The rôle of wild perennial hosts in preserving the virus of mosaic through the winter has not been fully established, but they must be regarded as a potential source of danger, and our studies are justified on these grounds. In the South, even the annual grasses may be found in the growing state during the whole year. Sugar cane itself is a perennial, and the virus of mosaic is known to survive the winter in the stubble, as the ratoon plants invariably come up diseased in the spring if the plants of the previous year were infected. The attempt has been made in some regions to eradicate the disease by destroying all infected cane stools or by plowing out whole fields and planting to other crops for a year or more. The labor of course would be wasted if the disease were perpetuated in some unobserved wild perennial host. This method of eradication has been successful, however, in at least one sugar-cane region, where the disease was early observed and prompt and vigorous measures were taken to stamp it out. This region is the peninsular section of Florida where the disease no longer exists to our knowledge.

More than 40 grasses have been tested experimentally, either by artificial inoculation or by means of insect inoculation or both.

[1] Accepted for publication Dec. 1, 1921.

[2] Reference is made by number (italic) to "Literature cited," p. 262.

EXPERIMENTAL INOCULATIONS

As a result of these experiments it has been conclusively proved that at least 13 species of grasses are subject to attack by the identical virus causing sugar-cane mosaic. Undoubtedly there are many more, but with the limited space at the disposal of the writers it was impossible to carry on the work on a larger scale. Species which have thus far given negative results may in the future prove to be susceptible. Many factors have entered which no doubt have diminished the chances for infection following these inoculations. Optimum growing conditions for many of the grasses do not prevail at Washington. Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes. Insect carriers became parasitized by other insects or died as a result of unfavorable conditions in the greenhouse, and they were not available or were not in a normal state when the plants were in the best condition for inoculation. In other words, the positive results attained must naturally carry more weight than the negative results, and in listing grasses as not susceptible it is merely meant that they did not become infected in these experiments.

Though it is possible that there is more than one mosaic disease affecting grasses, and there is some reason for believing this to be so, this paper is concerned with one only, the well-known type which has recently proved so destructive to sugar cane in Porto Rico and the southern United States.

The plants tested and found susceptible are shown in the following list:

Cultivated crops:
- Perennial—
  - Sugar cane.....*Saccharum officinarum.*
- Annuals—
  - Corn.....*Zea mays.*
  - Sorghum.....*Holcus sorghum.*
  - Pearl millet.....*Pennisetum glaucum.*

Ornamentals:
- Perennial—
  - Eulalia.....*Miscanthus sinensis.*

Wild grasses:
- Perennial—
  - Wild sugar cane.....*Saccharum narenga* (S. P. I. No. 38332).[3]
- Annuals—
  - Bull-grass.....*Paspalum boscianum.*
  - Crab-grass.....*Syntherisma sanguinalis.*
  - Yellow foxtail.....*Chaetochloa lutescens.*
  - Giant foxtail.....*Chaetochloa magna.*
  - Barnyard grass.....*Echinochloa crusgalli.*
  - Panicum.....*Panicum dichotomiflorum.*
  - Brachiaria.....*Brachiaria platyphylla.*

[3] A variety of *Saccharum narenga* is cultivated in China, according to Mr. Frank N. Meyer

The grasses tested with negative results are given in the following list:

Cultivated crops:
- Perennials—
  - Madake bamboo.....*Phyllostachys quilioi.*
  - Edible bamboo.....*Phyllostachys pubescens.*
  - Para grass.....*Panicum barbinode.*
  - Napier grass.....*Pennisetum purpureum var.*
  - Merker grass..... Do.
  - Australian giant-grass..... Do.
  - Johnson grass.....*Holcus halepensis.*
- Annuals—
  - Wheat, Power Fife (C. I. 3697).....*Triticum aestivum.*
  - Oats, Swedish Select (C. I. 134).....*Avena sativa.*
  - Rye, Von Runker No. 2 (C. I. 174).....*Secale cereale.*
  - Barley, Marionet.....*Hordeum vulgare.*
  - Rice, Blue Rose (C. I. 1962).....*Oryza sativa.*
  - Teosinte.....*Euchlaena mexicana.*
  - Redtop.....*Agrostis palustris.*
  - Timothy.....*Phleum pratense.*
  - Bluegrass.....*Poa pratensis.*
  - Ragi millet.....*Eleusine coracana.*

Ornamentals:
- Perennials—
  - Variegated Eulalia.....*Miscanthus sinensis variegatus.*
  - Do.....*Miscanthus sinensis zebrinus.*
- Annual—
  - Job's-tears.....*Coix lachryma-jobi.*

Wild grasses:
- Perennials—
  - Broom sedge.....*Andropogon virginicus.*
  - Broom sedge.....*Andropogon elliottii.*
  - Little bluestem.....*Andropogon scoparius.*
  - Indian reed.....*Sorghastrum nutans.*
  - Gama grass.....*Tripsacum dactyloides.*
- Annuals—
  - Green foxtail.....*Chaetochloa viridis.*
  - Red sprangletop.....*Leptochloa filiformis.*

It is noticeable that although plants have been selected for experiment from the entire grass family, the ones which proved susceptible are without exception confined to the tribes Paniceae, Andropogoneae, and Tripsaceae. In his phylogenetic arrangement of the genera of grasses, Hitchcock (*9*) divides the grass family into 13 tribes and places the 3 tribes here mentioned together as representing the highest type of development. It is significant that this arrangement, based on morphological characters, should prove so regular with respect to susceptibility to this disease. Since it is to be expected that closely related species are more likely to be affected by the same diseases than species located remotely from one another, the relationships are corroborated in an interesting and novel manner.

The possibility of the existence of more than one type of mosaic among the grasses has been mentioned. A type of mottling in the edible bamboo (*Phyllostachys pubescens*) from China resembles our mosaic very strikingly. It was first observed in one plant out of a lot of four being held in the detention house at Washington. Subsequently two other plants became affected. The writers have no experimental evidence that this is an infectious disease. It was found impossible to infect this species of bamboo with the sugar-cane mosaic. Furthermore, it is not nearly related to any other grass found susceptible to our mosaic but stands at the extreme opposite end of the list of tribes of the grass family.

A specific mosaic of *Nicotiana viscosum* distinct from the mosaic of *N. tabacum* has been reported by Allard (*1*), who states that the mosaic of

N. viscosum is more difficult to transmit artificially than the common tobacco mosaic. *Datura stramonium* was the only solanaceous plant found susceptible to both mosaic diseases. Allard suggests that results of inoculations with tobacco mosaic by various European investigators which are inconsistent with his own may be due to the existence of different types of mosaic in the Solanaceae.

COMPOSITE RECORD AND RESULTS OF EXPERIMENTS

The technic of the work here reported has been exactly the same as that described in a previous paper (*3*). All inoculations were made with one-half to 2 cc. of cell sap, obtained by squeezing young stalks in a powerful press under mineral oil. The virus or healthy sap was injected near the growing point by means of Leur all-glass hypodermic syringes provided with Yale 24-gauge needles. The results of the experiments are given in support of the facts presented in the preceding pages. In order to save space, composite records of the experiments are shown in tabular form.

Table I includes tests made for artificial transmission.

TABLE I.—*Composite record of tests of the artificial transmission of grass mosaic*

Species.	Number of plants.	Date inoculated.	Inoculum.	Results.	
				Date examined.	Condition.
Saccharum officinarum var. Louisiana Purple.	4	Oct. 4, 1920	Virus from sugar cane variety B6450.	Oct. 18, 1920	3 mosaic.
Do	4	.....do.....	Sap from healthy sugar variety B6450.	Nov. 15, 1920	All healthy.
Do	4	.....do.....	Virus from B6450 passed through Berkefeld filter.	Oct. 18, 1920	3 mosaic.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 0.2 per cent phenol.	.....do.....	1 mosaic.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 0.05 per cent $CuSO_4$.	Nov. 15, 1920	All healthy.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 5 per cent formalin.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 mixed with 1 per cent Carrol Dakin solution	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 mixed with 0.1 per cent $HgCl_2$.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/100 with distilled water	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/1,000 with distilled water.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/10,000 with distilled water.	.....do.....	Do.
Do	8	Aug. 4, 1920	Virus from Louisiana Purple.	Aug 28, 1920	8 mosaic.
Zea mays var. U. S. Selection No. 182.	3	.....do.....	.....do.....	Sept. 29, 1920	All healthy.
Holcus sorghum	2	.....do.....	.....do.....	Aug. 28, 1920	2 mosaic.
Syntherisma sanguinalis.	6	.....do.....	.....do.....	.....do.....	5 mosaic.
Coix lachryma-jobi	2	.....do.....	.....do.....	Sept. 29, 1920	Both healthy.
Pennisetum glaucum	6	.....do.....	.....do.....	Aug. 28, 1920	6 mosaic.
Saccharum officinarum var. Louisiana Purple.	8	.....do.....	Sap from healthy sugar cane, variety Louisiana Purple.	Sept. 29, 1920	All healthy.
Zea mays	3	.....do.....	.....do.....	.....do.....	Do.
Holcus sorghum	2	.....do.....	.....do.....	.....do.....	Do.
Syntherisma sanguinalis.	6	.....do.....	.....do.....	.....do.....	Do.
Coix lachryma-jobi	2	.....do.....	.....do.....	.....do.....	Do.
Pennisetum glaucum	6	.....do.....	.....do.....	.....do.....	Do.

It will be noticed that in the experiments the virus was treated in various ways. In at least one experiment virus which had been passed through a Berkefeld filter [4] caused infection in 75 per cent of the plants inoculated. Other similar experiments have failed to give such convincing results, but owing to the fact that control plants inoculated with untreated virus also show a low percentage of infection, indicating a less potent virus, the writers are inclined to attach considerable importance to the successful experiment and believe that the virus will pass through certain diatomaceous earth filters.

In some experiments the virus was shaken in bottles with various bacteriacidal chemicals one hour before being injected into the plants. With one exception none of the virus treated in this way caused infection. One plant in a series of four inoculated with virus treated with weak phenol solution became mosaic in 14 days. The same lot of virus diluted with distilled water in various proportions gave negative results. Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later. The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

The results of the experiments on insect transmission of the disease are given in Table II. "Virulent" insects were obtained from mosaic sugar cane or from mosaic sorghum artificially inoculated with virus from sugar cane. "Nonvirulent" insects were from healthy cane or sorghum. These experiments were performed in insect-proof compartments in a mosaic-infested greenhouse.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Saccharum officinarum var. Louisiana Purple.	3	Feb. 4, 1920	Virulent	*Aphis maidis*	Feb. 28, 1920	2 mosaic.
Do	3	do	Nonvirulent	do	Mar. 14, 1920	All healthy.
Do	6	do	Virulent	*Draeculacephala mollipes.*	do	Do.
Do	6	do	Nonvirulent	do	do	Do.
Zea mays var. U. S. Selection No. 182.	6	Mar. 12, 1920	Virulent	*Aphis maidis*	Apr. 6, 1920	4 mosaic.
Do	6	do	Nonvirulent	do	May 1, 1920	All healthy.
Do	20	Mar. 30, 1920	Virulent	do	May 28, 1920	15 mosaic.
Do	20	do	Nonvirulent	do	do	All healthy.
Triticum sativum var. Power Fife.	50	May 20, 1920	Virulent	do	Aug. 1, 1920	Do.
Do	50	do	Nonvirulent	do	do	Do.
Hordeum vulgare var. Marionet.	50	do	Virulent	do	do	Do.
Do	50	do	Nonvirulent	do	do	Do.
Secale cereale var. Von Runker No. 2.	50	do	Virulent	do	do	Do.
Do	50	do	Nonvirulent	do	do	Do.
Avena sativa var. Swedish Select.	50	do	Virulent	do	do	Do.
Do	50	do	Nonvirulent	do	do	Do.

[4] This was a rather coarse filter. When immersed in water it withstood a pressure of only 3 pounds before passing bubbles.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Oryza sativa var. C. I. No. 1962.	50	May 20, 1920	Virulent	*Aphis maidis*.	Aug. 1, 1920	All healthy.
Do	50	do	Nonvirulent	do	do	Do.
Eleusine coracana var. S. P. I. No. 43190.	50	do	Virulent	do	do	Do.
Do	50	do	Nonvirulent	do	do	Do.
Agrostis palustris	40	May 15, 1920	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Poa pratensis	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Holcus halepensis	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Phleum pratense	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	6	do	Virulent	do	June 15, 1920	All mottled; not clear case of mosaic.
Do	6	do	Nonvirulent	do	do	All healthy.
Miscanthus sinensis	16	May 20, 1920	Virulent	do	July 15, 1920	5 mosaic.
Do	6	do	Nonvirulent	do	do	All healthy.
Pennisetum purpureum.	4	May 30, 1920	Virulent	do	June 30, 1920	Do.
Do	4	do	Nonvirulent	do	do	Do.
Merker grass	4	do	Virulent	do	do	Do.
Do	4	do	Nonvirulent	do	do	Do.
Australian giant grass.	4	do	Virulent	do	July 6, 1920	Do.
Do	4	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	10	June 1, 1920	Virulent	do	July 29, 1920	6 mosaic.
Do	10	do	Nonvirulent	do	do	All healthy.
Zea mays var. U. S. Selection No. 182.	50	do	Virulent	do	July 28, 1920	2 mosaic.
Do	50	do	Nonvirulent	do	do	All healthy.
Paspalum boscianum.	3	July 31, 1920	Virulent	do	Aug. 31, 1920	3 mosaic.
Do	3	do	Nonvirulent	do	do	All healthy.
Saccharum officinarum var. Louisiana Purple.	10	Aug. 3, 1920	Virulent	do	Aug. 28, 1920	3 mosaic.
Do	10	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Zea mays	10	do	Virulent	do	Aug. 28, 1920	3 mosaic.
Do	10	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Holcus sorghum	5	do	Virulent	do	Aug. 28, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	5	do	Virulent	do	Aug. 28, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Miscanthus sinensis.	2	do	Virulent	do	Oct. 3, 1920	2 mosaic.
Do	2	do	Nonvirulent	do	do	All healthy.
Saccharum officinarum var. Louisiana Purple.	10	do	Virulent	*Kolla similis*	Sept. 15, 1920	Do.
Do	10	do	Nonvirulent	do	Sept. 9, 1920	Do.
Zea mays	10	do	Virulent	do	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Holcus sorghum	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Miscanthus sinensis.	2	do	Virulent	do	do	Do.
Do	2	do	Nonvirulent	do	do	Do.
Saccharum officinarum var. Louisiana Purple.	10	do	Virulent	*Draeculacephala molipes.*	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Zea mays	10	do	Virulent	do	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Holcus sorghum	5	do	Virulent	do	Sept. 15, 1920	Do.
Do	5	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Syntherisma sanguinalis.	5	Aug. 3, 1920	Virulent	*Draeculacephala molipes.*	Sept. 15, 1920	All healthy.
Do	5	do	Nonvirulent	do	do	Do.
Miscanthus sinensis.	2	do	Virulent	do	do	Do.
Do	2	do	Nonvirulent	do	do	Do.
Brachiaria platykylla.	7	July 13, 1920	Virulent	*Aphis maidis*	Aug. 13, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	do	All healthy.
Syntherisma sanguinalis.	2	Aug. 16, 1920	Virulent	do	Sept. 20, 1920	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy.
Paspalum boscianum.	2	do	Virulent	do	do	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy
Leptochloa filiformis.	1	Aug. 17, 1920	Virulent	do	do	Healthy.
Do	1	do	Nonvirulent	do	do	Do.
Saccharum officinarum.	3	Sept. 2, 1920	Virulent	*Draeculacephala molipes.*	Nov. 1, 1920	All healthy.
Zea mays	3	do	do	do	do	Do.
Holcus sorghum	2	do	do	do	do	Do.
Coix lachryma-jobi	2	do	do	do	do	Do.
Syntherisma sanguinalis.	2	do	do	do	do	Do.
Miscanthus sinensis.	2	do	do	do	do	Do.
Leptochloa filiformis.	6	do	do	*Aphis maidis*	Nov. 18, 1920	Do.
Saccharum officinarum.	10	Oct. 1, 1920	Exposed on ledge. [a]		Feb. 25, 1921	9 mosaic.
Holcus sorghum	15	Feb. 3, 1921	do		Apr. 2, 1921	6 mosaic.
Chaetochloa lutescens.	3	do	do		do	3 mosaic.
Syntherisma sanguinalis.	4	do	do		do	All healthy.
Paspalum boscianum.	6	do	do		do	6 mosaic.
Saccharum officinarum.	3	do	Virulent	*Draeculacephala molipes.*	May 6, 1921	All healthy.
Do	3	do	Nonvirulent	do	do	Do.
Echinochloa crusgalli.	3	Apr. 7, 1921	Exposed on ledge. [a]		May 25, 1921	2 mosaic.
Miscanthus sinensis zebrina.	3	Apr. 13, 1921	do		June 15, 1921	Healthy.
Miscanthus sinensis variegata.	4	do	do		do	Do.
Sorghastrum nutans	3	do	do		do	All healthy.
Chaetochloa magna.	4	do	do		do	2 mosaic.
Paspalum boscianum.	4	May 25, 1921	Virulent	*Aphis maidis*	June 20, 1921	4 mosaic.
Chaetochloa magna.	2	do	do	do	do	2 mosaic.
Chaetochloa lutescens.	8	do	do	do	do	All healthy.
Andropogon elliottii.	6	do	do	do	do	Do.
Andropogon virginicus.	6	do	do	do	do	Do.
Andropogon scoparius.	6	do	do	do	do	Do.
Syntherisma sanguinalis.	3	do	do	do	do	2 mosaic.
Brachiaria platyphylla.	4	do	do	do	do	All healthy.
Panicum dichotomiflorum.	2	do	do	do	do	2 mosaic.
Sorghastrum nutans.	2	do	do	do	do	Both healthy.

[a] These plants were merely exposed to natural infection by being placed on a ledge in a greenhouse containing infected plants with which they were not in direct contact.

The percentage of plants infected in both artificial-inoculation and insect-transmission experiments is not high in many cases, but the results are conclusive owing to the conditions under which all experiments were performed. All grasses for experimental purposes were grown in a greenhouse at Washington hundreds of miles from any known

cases of natural infection. When suitable for inoculation they were removed to another greenhouse where the inoculations were performed. In practically every case equal numbers of plants were inoculated with nonvirulent juice as controls. Insect-transmission experiments were conducted in cages in a third greenhouse with plant material raised in the greenhouse first mentioned. In these experiments also equal numbers of control plants were placed in adjoining cages. No case of mosaic has occurred among the control plants in any experiment performed.

In some experiments a method of handling aphids was developed by which the tedious operation of lifting individual insects with a camel's hair brush was eliminated. Small bits of infected sorghum leaves covered with the insects were clipped off with scissors and placed on or tied to the plants to be tested. Similar pieces of leaves from which the insects had been carefully removed were tied to a series of control plants, and healthy leaves with nonvirulent aphids were tied to a second series of control plants. Although it is necessary to run two sets of control plants in order to check all factors properly, this method was found to be of great convenience. The experiments noted in Table III were performed in the manner described.

TABLE III.—*Results of a test of the insect transmission of grass mosaic by the new method.*

Species.	Number of plants.	Date exposed.	Treatment.	Results.	
				Date examined.	Condition.
Saccharum officinarum var. Louisiana Purple	3	Aug. 3, 1920..	Aphids on mosaic leaf.....	Aug. 24, 1920.	2 mosaic.
Do	3	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	All healthy.
Do	3	.....do.....	Aphids on healthy leaf.....	.....do.....	Do.
Zea mays	3	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	2 mosaic.
Do	3	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	All healthy.
Do	3	.....do.....	Aphids on healthy leaf.....	.....do.....	Do.
Holcus sorghum	2	.....do.....	Aphids on mosaic leaf.....	Aug. 26, 1920.	Both mosaic.
Do	2	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Both healthy.
Do	2	.....do.....	Aphids on healthy leaf.....	.....do.....	Do.
Miscanthus sinensis	1	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	Mosaic.
Do	1	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Healthy.
Do	1	.....do.....	Aphids on healthy leaf.....	.....do.....	Do.
Syntherisma sanguinalis.	2	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	Both mosaic.
Do	2	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Both healthy.
Do	2	.....do.....	Aphids on healthy leaf.....	.....do.....	Do.

Although a considerable number of species of insects have been tested for their ability to act as carriers of grass mosaic, it has been demonstrated only for *Aphis maidis* (*A. adusta*) by the writers. Our results on the transmission of the disease with this insect have been corroborated by Ledeboer (*13*),[5] who has also succeeded in transmitting it by the use of *A. sacchari*. Owing to its abundance and omnivorous habits, *A. maidis* has been found very convenient for use in ascertaining the susceptibility to mosaic of various grasses. Several grasses, notably corn and millet, have been found by the writers to be very difficult or even impossible to infect by artificial inoculation, but it is comparatively easy to bring about infection by the use of virulent corn aphids. The corn aphids, of course, do not feed on all the grasses equally well, and on some it was found quite impossible to establish them. They are not abundant on sugar cane.

[5] Since this paper was prepared, nearly two years ago, the work reported in Ledeboer's preliminary report has appeared: WILBRINK, G. "EEN ONDER OEK NAAR DE VERBREIDING DER GELESTREPENZIEKTE DOOR BLADLUIZEN." *In* Archief Suikerindus. Nederl.-Indië, Meded. v. h. proefst. v. d. Javasuikerindus. 1922, no. 10, p. 413–456. Dr. Wilbrink confirms *A. maidis* as a vector of mosaic but not *A. sacchari*.

A. maidis has been reported on sugar cane, however, (*5, 8, 18*)[6], and on other crops from many sugar-cane regions.

While it is usually difficult to establish *Aphis maidis* on sugar cane in the greenhouse by transplanting them from other grasses, they sometimes migrate naturally to the cane and are found on it in large numbers. One of the worst infestations of any grass by *A. maidis* ever seen by the writers was on sugar-cane seedlings about 5 months old. It was brought to our attention by Dr. B. T. Galloway, who was growing the seedlings in one of the Washington greenhouses. In some of our experiments where *A. maidis* failed to become established and disappeared within 2 or 3 days, the plants nevertheless were infected by them and showed symptoms after the usual incubation period of 14 to 20 days. Corn, sorghum, and pearl millet are favorite food plants for *A. maidis*, which is frequently found on them in enormous numbers. The insect is an ideal carrier in the case of these three crops. Several species of leafhoppers have been held under suspicion as vectors of grass mosaic on account of strong indirect evidence, but no positive proof of such capacity on their part was developed.

SOME ECONOMIC ASPECTS OF GRASS MOSAIC

TESTS WITH CANE VARIETIES OF THE NORTH INDIA TYPE

Practically all of the well-known varieties of sugar cane are susceptible to grass mosaic. Prof. F. S. Earle noticed, however, that the so-called Kavangire, a variety of the slender North India type, was not affected under conditions favorable to the transmission of the disease (*15*). Later observations have indicated that the Uba, grown extensively in Natal, and Cayana No. 10, in the sirup sections of this country, are apparently immune. These varieties are of the same type, and Prof. Earle has declared that Kavangire is identical with the old well-known Uba (*6*). A collection of varieties of this type from various parts of the world was brought together in Washington to determine whether immunity to mosaic is characteristic of the whole group. They were placed in a greenhouse exposed to natural infection, with the results indicated in Table IV.

TABLE IV.—*Susceptibility to the mosaic disease of the varieties of the North India type of sugar cane*

Variety.	Date exposed.	Result.	
		Date examined.	Condition.
Uba	Jan. 6, 1921	July 1, 1921	All healthy.
Kavangire	do	do	Do.
Cayana No. 10	do	do	Do.
Saccharum narenga (S.P.I. No. 38332).	do	Jan. 25, 1921	All mosaic.
Khera (S.P.I. No. 33242)	do	do	Do.
Merthi (S.P.I. No. 33243)	do	July 1, 1921	All healthy.
Kinar (S.P.I. No. 33245)	do	do	Do.
Chikusho (S.P.I. No. 29106)	do	Jan. 25, 1921	All mosaic.
Var. from Kagawa Ken (S.P.I. No. 29107).	do	do	Do.
Kikaigashima (S.P.I. No. 29108)	do	do	Do.
Oshima (S.P.I. No. 29109)	do	July 1, 1921	All healthy.
Chikucha (S.P.I. No. 30464)	do	Jan. 25. 1921	All mosaic.

[6] Mr. A. C. Baker reports *Aphis maidis* on sugar cane in the quarantine greenhouse of the Federal Horticultural Board at Washington, March 11, 1920 (in letter to the writers, December 2, 1921), and Mr. Geo. W. Wolcott reports finding this species on sugar cane in Porto Rico (in letter to Dr. C. O. Townsend, December 30, 1921).

With the exception of the plants labeled *Saccharum narenga*, these varieties are all very similar, and some of them may be identical. They are of the slender North India type and probably have a common origin, but this experiment proves that not all varieties of this type are immune to mosaic. Varieties from India, China, and Japan proved to be susceptible. They are apparently scarcely injured by the disease. The leaf symptoms are much less conspicuous than in the thick-stalked varieties of sugar cane, and there is no evidence of stunting. In this respect they are like the well-known Java seedling varieties resulting from crosses between the Chunnee ♂ and Striped Preanger ♀ and between Chunnee ♂ and Black Cheribon ♀.

TESTS WITH CORN VARIETIES

That some varieties of corn are severely injured by grass mosaic was shown by the senior writer in 1920 (*4*). In 1921 a large number of corn varieties were tested in southern Georgia for immunity or resistance to the disease. The first experiment included 40 varieties of field, sweet, and pop corn from all of the corn sections of the United States.[1] About 25 plants of each variety were grown near the center of a field of first ratoon Louisiana Purple sugar-cane plants, more than half of which were mosaic. Seeds were planted in the field on April 15, 1921. On July 15, 1921, the plot was examined and notes were taken on the percentage of infected plants in each variety. The results are given in Table V.

TABLE V.—*Results of tests for resistance to mosaic of varieties of corn planted on April 15, 1921*

Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.	Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.
Native	Georgia	20	Clarage (U. S. Selection No. 125).	Maryland	0
Pope Prolific	Florida	20	Boone County (U. S. Selection No. 159).	Nebraska	0
U. S. Selection No. 165	Texas	35	St. Charles (U. S. Selection No. 202).	do	0
U. S. Selection No. 170	do	40	Lancaster Surecrop	Illinois	0
Laguna	do	10	U. S. Selection No. 160	California	15
Brazos	do	5	Orange County Prolific	do	15
Arlington Prolific	Mississippi	17	U. S. Selection No. 204	South Dakota	10
Red Cob	do	10	Northwestern	North Dakota	0
Millpond Prolific	Georgia	20	Gehu	do	0
Whatley Prolific	do	15	Pearl	do	0
Gerrick	South Carolina	12	U. S. Selection No. 133	Wisconsin	0
U. S. Selection No. 201	Arkansas	12	Hall Gold Nugget (U. S. Selection No. 193).	New York	0
Cuban Yellow	Florida	20	Arlington Peruvians	Virginia	15
Station Yellow	Alabama	0	Pueblo Black	New Mexico	0
Singleton	Texas	0	White Rice	Virginia	4
Huffman	Tennessee	10	Yellow Pearl	do	4
U. S. Selection No. 230	Virginia	5	Hull-less	Michigan	0
Boone County (U. S. Selection No. 119).	do	10	Golden Bantam	Virginia	0
U. S. Selection No. 120	do	0	Country Gentleman	do	0
U. S. Selection No. 182	do	10			
Woodburn (U. S. Selection No. 77).	Ohio	0			

Twenty-three of the 40 varieties became affected with mosaic in this experiment. All varieties from the Southern States excepting 4, which later proved susceptible, were more or less affected by the disease; but

[1] The seed of these varieties was furnished through the courtesy of the Office of Cereal Investigations, U. S. Department of Agriculture.

the varieties from Northern and Western States were conspicuously free from it. This result may be due in part to the fact that the northern and western varieties were subnormal in vigor. They were for the most part only half as tall as the southern varieites. It is now well known that a slight shortening of the accustomed length of day may check vegetative growth and hasten maturity in some varieties of plants (7), and this fact may account for the subnormal development of these varieties. It has been our experience that stunted plants are more difficult to infect experimentally than normal ones. The explanation for the low percentage of infection in the varieties which were out of their proper environment may therefore be due to this fact rather than to any innate character of resistance.

A second experiment on a much larger scale with southern varieties of field corn was started on May 15, one month after the first experiment. About 3 acres of corn, approximately equally divided among 17 varieties, was planted in a field immediately adjacent to badly diseased first ratoons of Louisiana Purple sugar cane. On July 15 the whole planting was carefully examined and a large proportion of all varieties was discovered to be already affected by the disease. The percentage of diseased plants on that date is given in Table VI.

TABLE VI.—*Results of tests for resistance to mosaic of varieties of southern field corn planted on May 15, 1921*

Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.	Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.
Native	Georgia	15	Whatley Prolific	Georgia	40
Pope Prolific	Florida	75	Gerrick	South Carolina	50
U. S. Selection No. 165	Texas	30	U. S. Selection No. 201	Arkansas	60
U. S. Selection No. 170	do	50	Cuban Yellow	Florida	45
Laguna	do	24	Station Yellow	Alabama	66
Brazos	do	40	Singleton	Texas	60
Arlington Prolific	Mississippi	50	U. S. Selection No. 230	Virginia	45
Red Cob	do	25	Boone County (U. S. Selection No. 119).	do	70
Millpond Prolific	Georgia	85			

A much higher percentage of infection was found in these plants than in the same varieties planted one month earlier. All of the corn was heavily infested with *Aphis maidis*, but no other insect was noticeably abundant.

In order to obtain data on the damage inflicted by this disease, 10 each of mosaic and apparently healthy plants in each variety were tagged for identification at harvest time. It is realized that many plants marked "healthy" on July 15 may have become infected before harvest, so that any decrease in yield indicated by this method does not represent the real extent of the loss sustained. The loss due to mosaic is without question greater than that indicated by our data. All tagged plants with legible inscriptions were harvested on September 22, and the ears were examined and weighed. The number of ears, total weight, and average weight of ears of healthy and mosaic plants for each variety are given in Table VII.

TABLE VII.—*Weight of healthy and mosaic ears of corn varieties planted May 15, 1921*

Variety.	Source of seed.	Condition.	Ears on 10 plants.	Weight.		Decrease due to mosaic.
				Total.	Average.	
			Number.	*Gm.*	*Gm.*	*Per cent.*
Native	Georgia	Healthy	5	535	107	.....
Do	do	Mosaic	7	470	67.1	37.3
Pope Prolific	Florida	Healthy	8	822	102.7	.....
Do	do	Mosaic	5	402	80.4	21.8
U. S. Selection No. 165	Texas	Healthy	4	665	166.2	.....
Do	do	Mosaic	9	780	82.2	50.6
U. S. Selection No. 170	do	Healthy	6	995	165.8	.....
Do	do	Mosaic	6	830	138.3	16.6
Laguna	do	Healthy	5	765	153.0	.....
Do	do	Mosaic	5	735	147.0	4.0
Brazos	do	Healthy	6	1,115	186.0	.....
Do	do	Mosaic	7	815	116.4	37.5
Arlington Prolific	Mississippi	Healthy	10	1,100	110.0	.....
Do	do	Mosaic	6	470	78.2	29.0
Red Cob	do	Healthy	4	555	138.7	.....
Do	do	Mosaic	5	640	128.0	7.8
Millpond Prolific	Georgia	Healthy	7	750	107.1	.....
Do	do	Mosaic	10	595	59.5	44.5
Whatley Prolific	do	Healthy	7	790	112.8	.....
Do	do	Mosaic	11	880	80.0	29.1
Gerrick	South Carolina	Healthy	7	580	82.9	.....
Do	do	Mosaic	12	890	74.1	20.7
U. S. Selection No. 201	Arkansas	Healthy	8	910	113.5	.....
Do	do	Mosaic	6	518	86.3	24.0
Cuban Yellow	Florida	Healthy	5	815	163.0	.....
Do	do	Mosaic	12	1,140	95.0	41.8
Station Yellow	Alabama	Healthy	8	900	112.2	.....
Do	do	Mosaic	8	755	94.3	16.0
Singleton	Texas	Healthy	7	784	112.0	.....
Do	do	Mosaic	6	670	111.6	.4
U. S. Selection No. 230	Virginia	Healthy	2	318	159.0	.....
Do	do	Mosaic	3	317	102.3	35.7
Boone County (U. S. Selection No. 119)	do	Healthy	2	205	102.5	.....
Do	do	Mosaic	6	435	72.5	29.3

This data indicates that with corn, as with sugar cane, some susceptible varieties tolerate the disease without greatly injurious results, while other varieties are severely injured by it. Plate 1, B, illustrates a variety not noticeably injured by mosaic. It was pointed out in a former publication (*4*) that in the case of "White Creole" corn in Louisiana the ears of affected plants are not only stunted but they are poorly filled, some being quite sterile. The rows of kernels in affected ears are likely to be very irregular as compared with the straight parallel rows of normal ears. In nearly all varieties in the present experiments the same condition was apparent and is well illustrated in Plate 1, A, and also in Plates 2 to 4. It will be noticed that some of the ears marked "healthy" are small and some show irregularity in the rows, but by no means to the same degree as in the mosaic ears. This may be due partly to the fact previously noted that plants labeled healthy in the field may have become infected later. The writers believe that grass mosaic is a serious disease of corn where conditions are favorable for infection. Since the disease is apparently not transmitted in the corn seed, as will be briefly considered

later, the infection must come each year from some diseased perennial grass. In all sugar-cane plantations, corn is invariably used in the crop rotation, and one of the required conditions for infection of corn is present if the disease exists in the nearby cane.

Corn mosaic has now been reported from Porto Rico, the United States, Guam, the Hawaiian Islands, and Trinidad.

In a publication by Kunkel (*12*), just received from Hawaii, a very complete cytological investigation of corn mosaic is recorded. Certain large bodies found in the cells of diseased tissues are described and suggested as the possible cause of the disease. Such cell inclusions have been noticed and recorded by early investigators of mosaic, but this was not mentioned and possibly was overlooked by Kunkel. Practically identical bodies were carefully described and accurately illustrated by Iwanowski (*10*) in his researches on tobacco mosaic published nearly 20 years ago. The latter, however, was cautious about ascribing to these bodies any etiological significance merely on the basis of their association with the disease. The paper by Kunkel referred to above contains some data of value on susceptibility of corn varieties to mosaic. His presentation of the history of our knowledge of this disease in corn is very misleading. One of the present writers contributed the original paper on corn mosaic (*4*). This paper, which was based on careful observations and experiments established the type of disease, its infectious nature, its identity with sugar-cane mosaic, and the natural agents of transmission. Within its pages ample credit was given to two previous investigators for observation of a possibly similar condition in corn. Their observations were of a very indefinite nature and were summed up in the following words by the only one of the two who published on it (*16*):

> The trouble was ascribed to various causes by different people, *but it appears to be the same baffling general condition observed in Hawaii rather than any specific disease.*[8]

On the basis of this candid admission and other less reliable evidence Kunkel, perhaps unintentionally, leads the reader to believe that the disease was well known and well understood prior to the present writers' contribution.

FIELD OBSERVATIONS ON SORGHUM AND PEARL-MILLET MOSAIC

Although mosaic on sorghum was early noted by one of the writers in experiments at Washington (*2*) and afterwards proved to be identical with sugar-cane mosiac (*3*), it was not observed in the field until the summer of 1920. At that time many fields of sweet sorghum were noticed to be naturally infected in the vicinity of Cairo, Ga. All such fields were within half a mile of affected sugar cane. No accurate data were obtained on injury to sorghum, but the leaf symptoms were strongly marked, the nondevelopment of chlorophyll being about the same as in severely injured sugar cane, and the plants were decidedly stunted. It was noticed that when plants were infected soon after germinating, the injury was far greater than in the case of late infection. A few notes were taken on varieties affected and on the percentage of infected plants in various fields.

The variety Honey appears to be especially susceptible. Several fields of this variety, known locally as Sugar Drip, were observed to contain 6

[8] The italics are ours.

to 30 per cent of diseased plants. Fields of the so-called Texas-Ribbon variety (probably Gooseneck) contained from 0.5 to 15 per cent of affected plants, or uniformly less than the first-named sort under the same conditions. No infection was observed in a field of Early Amber, but this variety has proved to be susceptible under greenhouse conditions at Washington. A number of grain-sorghum varieties recently introduced from Africa were planted in a greenhouse at Washington exposed to natural infection, but they have showed no signs of the disease. About 50 large stools of mosaic sugar cane were present in this house, and *Aphis maidis* was abundant.

During the summer of 1921 a patch of pearl millet (*Pennisetum glaucum*), sold under the name Georgia cat-tail millet, was planted for soiling purposes at the Sirup Experiment Station, Cairo, Ga. On July 15 this planting was noticed to be severely attacked by mosaic. More than 50 per cent of the plants were affected and were noticeably smaller than healthy plants. This species also had been previously proved to be susceptible to grass mosaic under controlled conditions at Washington. (See Table II.)

FIELD OBSERVATIONS ON MOSAIC OF WILD GRASSES

Collections of wild grasses affected with mosaic have been made in Louisiana, Georgia, and Florida. In all cases the plants were found in or near affected sugar-cane fields. That the mosaic appearing naturally in the field in these species is identical with sugar-cane mosaic has been verified experimentally under controlled conditions. (See Tables I and II.) A list of mosaic grasses collected with dates and localities is given in Table VIII.

TABLE VIII.—*Collections of wild grasses affected with mosaic*

Species and locality.	Date of collection.	Species and locality.	Date of collection.
Syntherisma sanguinalis:		*Paspalum boscianum:*	
Cairo, Ga.	Sept. 11, 1919	Cairo, Ga.	Sept. 11, 1919
New Orleans, La.	Oct. 15, 1919	Marianna, Fla.	Aug. 16, 1920
Do.	Sept. 6, 1920	Cairo, Ga.	July 27, 1920
Chattahoochee, Fla.	Sept. 1, 1920	Reno, Ga.	July 29, 1920
Plaquemine, La.	Aug. 21, 1920	*Chaetochloa magna:*	
Marianna, Fla.	Aug. 14, 1920	Dade County, Fla.	Dec. 11, 1920
Reno, Ga.	July 31, 1920	*Brachiaria platyphylla:*	
		New Orleans, La.	Aug. 11, 1920
		Do.	Sept. 6, 1920

No complete record of individual observations on mosaic of wild grasses has been kept. The foregoing list is sufficient to show that mosaic of these species is common near affected sugar cane under natural conditions.

EXPERIMENTS ON SEED TRANSMISSION OF GRASS MOSAIC

It seems appropriate to include in the present paper a brief account of experiments to determine whether the disease is transmitted by means of seeds, because of the relation of such transmission to the infection of one species with inoculum from a different species. In regions where the vegetative parts of annual grasses are completely killed during winter,

the virus would be more likely to persist in seeds, if it survives at all, than in any other state. The possibility of its being carried over in the bodies of insect vectors is rather remote, since there is no evidence that such insects function as intermediate hosts, but rather as mechanical carriers, and as such they would not furnish the special conditions necessary for long-continued survival of the virus. This view is supported by our results with artificial inoculations, which proved that development of the virus within the body of an insect is not necessary. We have fairly conclusive proof that the virus does not survive in plant trash or soil, even in tropical countries. Our evidence that the virus is not carried over in seeds (*2*) has been strengthened by the results of subsequent experiments with corn, sorghum, and wild-grass seeds from mosaic parents, all of which gave rise to healthy plants. The results are shown in Table IX.

TABLE IX.—*Experiments to determine the transmission of disease by means of seeds from mosaic corn, sorghum, and wild grasses*

Species.	Date planted.	Number germinated.	Results.	
			Date examined.	Condition of seedlings.
Zea mays var. White Creole.	Nov. 30, 1920	44	Jan. 25, 1921	All healthy.
Zea mays var. U. S. Selection No. 182.	.....do.....	81	.....do.....	Do.
Zea mays var. White Creole.	Dec. 15, 1920	100	Feb. 15, 1921	Do.
Zea mays var. U. S. Selection No. 182.	.....do.....	90	.....do.....	Do.
Holcus sorghum var. Honey.	Sept. 15, 1919	181	Nov. 30, 1919	Do.
Do.....	Nov. 30, 1920	94	Jan. 26, 1921	Do.
Holcus sorghum var. Gooseneck.	.....do.....	75	.....do.....	Do.
Paspalum boscianum.....	Jan. 20, 1921	47	Mar. 12, 1921	Do.
Brachiaria platyphylla.....	.....do.....	52	.....do.....	Do.
Syntherisma sanguinalis.....	.....do.....	42	.....do.....	Do.

Observations by Vander Stok (*14*), Kobus (*11*), and Wilbrink and Ledeboer (*17*) in Java indicate that sugar-cane seedlings from mosaic parents are healthy at the start and remain so unless infected from outside sources in the usual way. All available evidence, therefore, points to the conclusion that in this disease, as in tobacco mosaic, the virus is not transmitted from generation to generation by means of seeds.

SUMMARY

(1) Thirteen species of grasses have been proved by inoculation experiments to be susceptible to the disease known as sugar-cane mosaic or, more properly, grass mosaic.

(2) Certain varieties of sugar cane belonging to the slender North India type, formerly regarded as immune, have proved susceptible to mosaic.

(3) Data on the yield of 17 varieties of southern field corn show a decrease in weight of 0.4 to 50.6 per cent, due to mosaic.

(4) Field observations indicate that natural infection of sorghum, pearl millet, crab-grass, bull-grass, *Chaetochloa magna*, and *Brachiaria platyphylla* is widespread near affected cane in the sugar-cane belt.

(5) All species tested for seed transmission of mosaic gave negative results.

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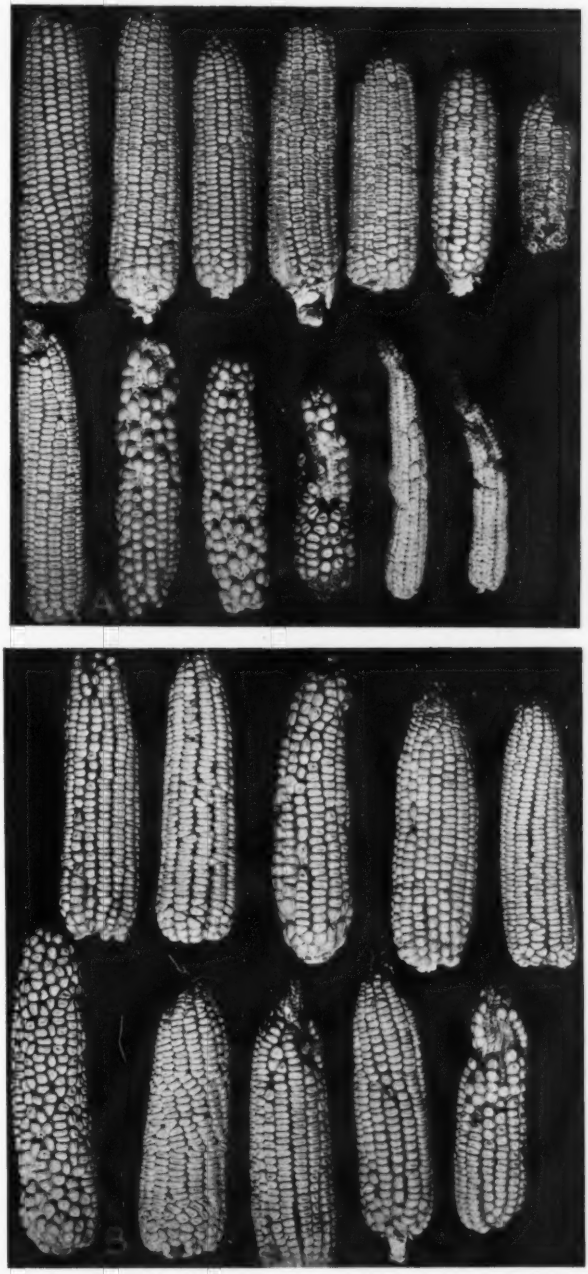
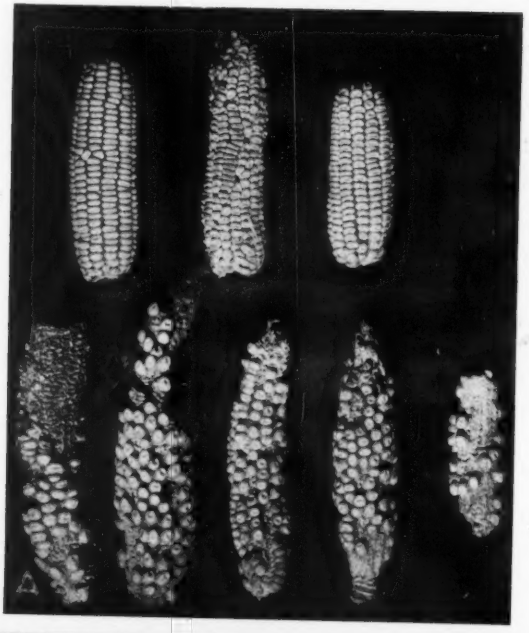
PLATE 1

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Arlington Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) Laguna corn, a resistant variety.




A

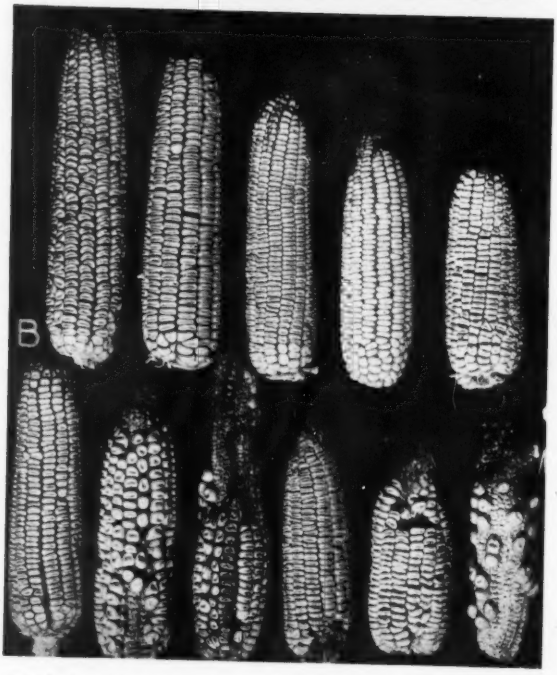

B

PLATE 2

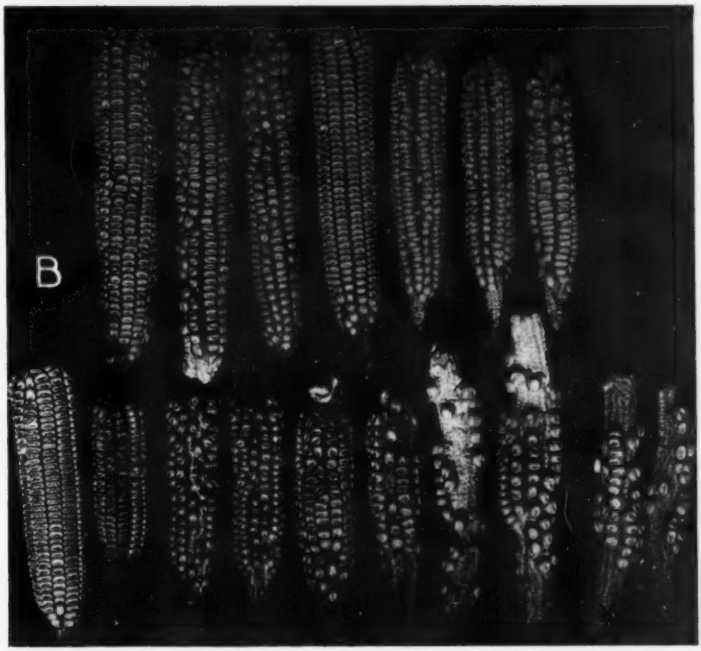
A.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 119.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 170.

PLATE 3

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Millpond Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 165.


B

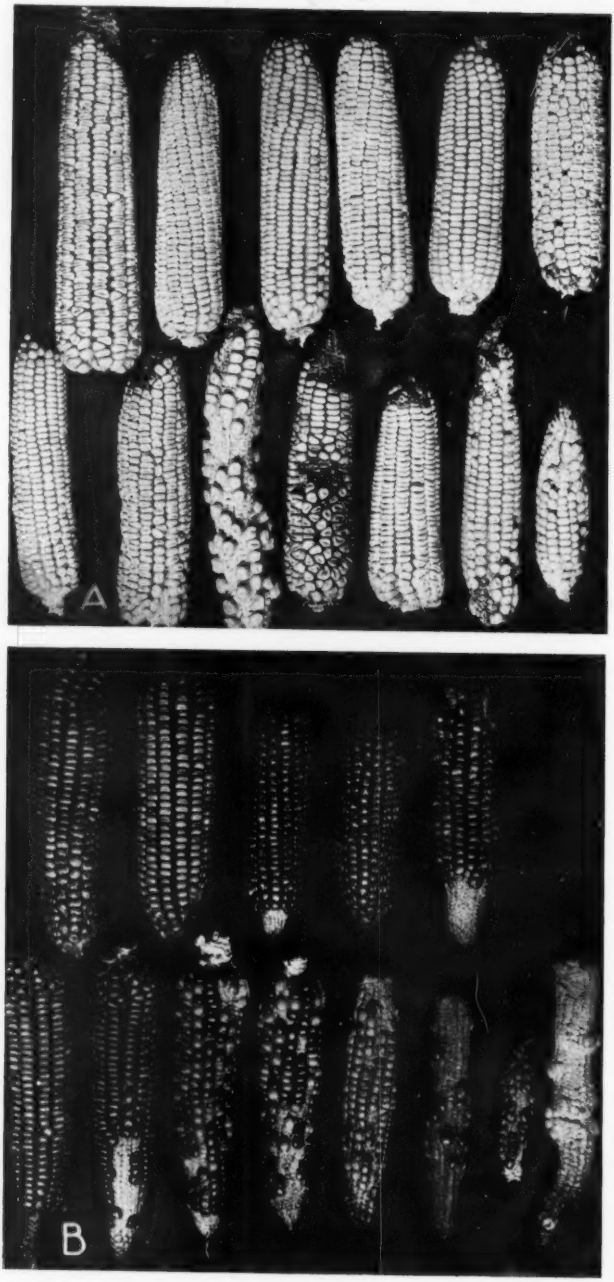

A
B

PLATE 4

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Brazos variety.
B.—Ears of healthy (upper row) and mosaic (lower row) corn of Cuban Yellow variety.

PROTEIN SYNTHESIS BY AZOTOBACTER [1]

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The metabolic process of protein synthesis is common to all microorganisms. The rate, form, and total quantity of protein synthesized, however, as well as the type of food substances available for synthesis, varies widely.

An idea of the relative protein content of microorganisms can be obtained from the results recorded by various investigators; but the multiplicity of the methods employed in the cultivation, collection, and analysis of microbial cells makes a comparative study difficult. A high nitrogen content for most bacteria is reported. If all of this nitrogen be considered as protein nitrogen, the protein content of such cells is large. Wheeler (*8*, *p.* 65),[2] whose results seem to be comparative for such analyses, reports a nitrogen content varying from 5.964 per cent to 11.765 per cent for 12 bacteria examined. The nitrogen content of yeasts is probably similar to that of bacteria. Nicolle and Alilaire (*5*) report a nitrogen content of 10.0 per cent for a Froberg yeast.

The high protein content of yeasts, their rapidity of growth, and their practical method of cultivation justify their wide exploitation as a food product within recent years. The value of such yeast food as a protein concentrate appears to be firmly established. During the recent war the demand for this yeast concentrate in Germany far exceeded the supply.

Interest in the yeast industry has been accentuated with the development of the present vitamine theory. The high content of water-soluble B vitamine in yeast cells has apparently opened a new field for its use.

Several factors tend to influence the practicability of utilizing microorganisms as a source of protein. Mechanically, the problem is dependent upon the development of practical methods for securing a maximum degree of growth, as well as means for the collection and care of the product. Economically, the yield, rate of growth, and the food requirements of the organism are important considerations. The substances used as food for the organism should be in the nature of waste products, and to justify the process the synthesized product must have a greater value than the substances used in its production. Finally, if the industry is to be of value, the product must have a nutritional value.

The high protein content of the Azotobacter cell and its relatively simple food requirements suggested the possibility of utilizing it as a means for synthesizing a protein which could be used either as a stock food or as a fertilizer. The employment of this organism for such a purpose appeared to offer some important advantages on account of its nitrogen-assimilating ability. This would necessitate the use of a solution having a carbohydrate only as the important constituent.

On the other hand, the apparent slow development of Azotobacter seemed to present a serious difficulty. However, it has been demonstrated

[1] Accepted for publication May 31, 1922.
Contribution No. 40 from the Bacteriological Laboratories of the Kansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 274.

that a prompt and heavy growth of Azotobacter can be secured by a vigorous aeration of a liquid culture in a manner similar to that used in stimulating yeast growth.

The experimental data which follow are merely a report of progress in an investigation having for its object the utilization of waste products by Azotobacter for protein synthesis.

METHOD OF CULTIVATION

A rapid and vigorous growth of Azotobacter was produced by passing a current of air continually through a culture solution. The method used was identical with that described in a previous publication (*14*).

Aeration was accomplished by attaching the culture flask equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the culture were reduced to a minimum by filtering the air through a sterile cotton filter and two or three flasks of sterile water. The air was thus filtered through cotton and rinsed in water before entering the culture flasks.

Several flasks of culture media could be easily aerated at the same time. Usually a cotton filter and a wash bottle were placed between the culture flasks. Little trouble occurred from contamination. A good grade of rubber tubing and tight fitting connections are a necessity, however. Flasks containing from 200 cc. to 2,500 cc. of medium were used. The quantity of air passed through the cultures was not measured, but a vigorous bubbling of the liquid was maintained continuously.

AZOTOBACTER CULTURES

Cultures of Azotobacter were isolated from samples of soil received from various parts of Kansas, Colorado, California, Iowa, and Mississippi. Flasks containing dextrose-Ashby or mannite-Ashby medium were inoculated with the soil samples. Upon the formation of the characteristic surface growth, Ashby agar plates were streaked. Dextrose-Ashby agar slants were inoculated from well-isolated colonies and repeated streakings of the cultures upon Ashby agar plates were made. A large number of the cultures were streaked consecutively from 1 to 12 generations. The utmost care was exercised in attempting to obtain and maintain pure cultures.

The inoculum used for seeding the medium was prepared by adding a portion of the emulsion from a young dextrose-Ashby culture to a flask containing dextrose-Ashby broth. This was aerated for two to four days. The quantity of this starter usually used for inoculating the experimental medium was 0.5 per cent to 1.0 per cent of the medium seeded. In all cases a morphological examination of the starter was made as a test for purity before use. The temperature for incubation was 30° C.

MEDIUM

The medium used in the following experiments, unless otherwise noted, was made from the following formula:

	Gm.
Tap water	1,000.0
K_2HPO_4	.5
$MgSO_4$	.2
NaCl	.2
Dextrose	10.0

The reaction of this solution was readjusted to a P_H of 7.0 to 7.4, filtered if necessary. The required quantities were placed in flasks and sterilized in the autoclav at 20 pounds pressure for 30 minutes.

METHODS OF ANALYSIS

Total nitrogen determinations and sugar analysis of the Azotobacter cultures were made at frequent intervals.

In all cases the total nitrogen was determined by the usual standard methods. Unless otherwise noted, 50 cc. portions of the medium in duplicate were used. The figures referred to in the tables denote the average of the duplicate analyses. The net gain in nitrogen is recorded in all cases, unless otherwise stated, as milligrams of nitrogen in each 100 cc. of medium. In other words, the figures refer to the quantity of nitrogen fixed per gram of dextrose, since each 100 cc. of medium contains this quantity of sugar.

Sugar determinations were made according to the method proposed by Shaffer (*9*). The copper resulting from the reduced Fehlings was determined by colorimeter readings. Duplicate readings were made each time, and the average of these was recorded. As a general rule, 50 cc. of the medium were used. This was diluted to 100 cc., and 20 cc. of this filtrate were used for reduction. The figures given, unless otherwise stated, refer to grams of dextrose per 100 cc. of medium.

COMPOSITION OF THE AZOTOBACTER CELL

The composition of the Azotobacter cell has been determined by several investigators. Gerlach and Vogel (*1*) report a protein content of 80 per cent.

Lipman (*2*) reports the nitrogenous composition of the Azotobacter membrane as 10.45 per cent total nitrogen, 6.39 per cent nonbasic nitrogen, 2.76 per cent basic nitrogen, and 0.98 per cent ammonia nitrogen. He observed that lead acetate precipitated practically all of this nitrogen in young or old cultures, while phototungstic or tannic acid would not precipitate nearly as much. He believed that in young cultures this nitrogen substance is in a soluble form and not precipitated by phosphotungistic acid, but as the culture grows older this soluble nitrogenous material is converted into an insoluble and more complex protein.

Stoklasa (*4*) cultivated Azotobacter in liquid cultures. The growth was collected on a filter and washed. The nitrogen content of the washed cells was 11.3 per cent.

Hoffmann and Hammer (*6*) studied the composition of Azotobacter and report analyses much lower in protein. The organism was grown on Ashby agar plates. The growth was scraped off, washed, and dried. The maximum protein content recorded was 17.75 per cent. They suggest that the wide difference in their results, as compared with those of other investigators, was due to the jellylike material which in liquid cultures is filtered out of solution. In their method this material is included in the analysis. This increases the total residue, and since this substance is thought to be carbohydrate, the total percentage of nitrogen is decreased. The phosphorus content (P_2O_5) varied from 2.51 per cent to 2.97 per cent. Increasing with the age of the culture. Stoklasa (*4*) reports the P_2O_5 content to be 4.93 per cent.

Omeliansky (*7*) grew Azotobacter on dextrose mineral salt agar for six days at a temperature of 30° C. An analysis of this growth is

reported as 6.63 per cent moisture, 4.16 per cent ash, and 12.92 per cent protein. This protein he considers similar to other plant proteins.

While the exact method of cultivation is not reported by all investigators, it appears in general that the nitrogen content of Azotobacter will vary according to the method of cultivation. The nitrogen of the growth scraped off Ashby agar media is reported much lower than in the residue filtered from the liquid cultures.

The composition of seven cultures of Azotobacter cultivated on dextrose-Ashby agar was determined in this laboratory. The growth at the end of two to four days incubation at 30° C. was scraped off the surface of the agar, air dried, and analyzed for the total nitrogen and moisture content. The results are given in Table I.

TABLE I.—*Nitrogen content of Azotobacter cells grown on Ashby agar*

Culture No.	Total nitrogen.	Moisture.
	Per cent.	*Per cent.*
12 B	3.48	9.12
232	3.78	8.55
6 A	3.78	10.00
5 B	3.09	6.89
1 B	3.74	8.10
216	4.55	4.69
10 B	3.74	9.48
Average	3.73	8.12

The percentage of total nitrogen noted in the seven cultures was uniform, averaging 3.73 per cent for all cultures and ranging from 3.48 per cent to 4.55 per cent. If this nitrogen were calculated as protein, the average protein content of the cultures would be 23.31 per cent.

An analysis of a composite sample of these cultures gave the following results: Moisture 8.58 per cent; total nitrogen 3.55 per cent; albuminoid nitrogen 1.89 per cent; ash 12.99 per cent; phosphorus 0.57 per cent; and potassium 1.43 per cent. These figures indicate that only 53.1 per cent of the total nitrogen is protein. This gives a protein content of 11.81 per cent instead of 23.31 per cent, as calculated from the total nitrogen.

The chemical composition of the growth obtained from liquid cultures was determined for comparison. Culture No. 232 was aerated from two to four days at 30° C. in the dextrose-Ashby medium. The growth of cells was obtained by centrifuging in a Sharples laboratory supercentrifuge. A composite sample taken from the growth of several culture flasks was used for analysis. The composition was: Moisture 2.67 per cent; total nitrogen 5.15 per cent; albuminoid nitrogen 4.89 per cent; ash 4.62 per cent; phosphorus 0.24 per cent; and potassium 1.2 per cent.

A comparison of this analysis with that obtained from the growth on Ashby agar reveals considerable difference. In the first place, a higher total nitrogen content is observed in the growth from the liquid culture. The most marked effect, however, is the high albuminoid content of the liquid culture, it being 94.9 per cent of the total nitrogen. This gives a protein content of 30.56 per cent, as compared with 11.81 per cent for the growth obtained from Ashby agar. The liquid culture produced a growth with a lower ash content than did the culture on agar.

SOURCES OF ENERGY

There is a wide variety of substances available as energy for Azotobacter which differ greatly in their value as sources of energy. Not only is there a difference in the value of the sources of energy for nitrogen fixation, but the efficiency of the same material for azofication varies, according to different investigators. This is illustrated in the work of Löhnis and Pillai (*3*), Hoffmann and Hammer (*6*), and Mockenridge (*10*). There appears to be, however, a rather uniform opinion that a mannite solution will furnish energy for the fixation of the largest quantity of nitrogen. As a result, mannite is the carbohydrate employed almost universally in azofication experiments.

As a preliminary study, the comparative value of various substances as sources of energy for Azotobacter was determined. One per cent of the test material was substituted for mannite in Ashby's medium containing calcium carbonate. Flasks of each medium were inoculated with a pure culture of Azotobacter and aerated at 30° C. for six days. The average quantity of nitrogen fixed per gram of test substance for the two cultures studied were as follows:

	Mgm.
Potassium acetate	8.0
Dextrose	7.8
Saccharose	7.7
Mannite	7.2
Molasses	3.0
Lactose	2.4

Dextrose has been preferred in this laboratory as a source of energy for studying the nitrogen-fixing ability of different cultures and their rate of fermentation. Quantitative determination can be easily made and, as shown by these experiments with aerated cultures, it is an efficient source of energy for azofication.

While no special endeavor has been made to study the nitrogen-fixing ability of different Azotobacter cultures with the object of classifying them on such a basis, over 20 strains have been studied. The nitrogen fixation for these cultures grown in dextrose medium has varied from 7.20 mgm. to 18.72 mgm. per gram of dextrose. Many of the same cultures have been repeatedly used for the past two years, and the variations in the quantity of nitrogen fixed at different times by the same cultures is as much as the difference between various cultures. This variation, or at least a large portion, may be attributed to the different intensities of aeration to which the cultures were submitted. The aim was to aerate all cultures alike, but such is impossible unless the aeration is mechanically controlled. This suggests that the optimum quantity of air necessary for the maximum growth of Azotobacter should be determined.

YIELD OF AZOTOBACTER GROWTH

Cultures of Azotobacter cultivated by vigorous aeration in a liquid medium will exhibit a vigorous growth within two to four days. The sugar is rapidly consumed, and a corresponding increase in the nitrogen content of the liquid is noted. If the culture produces pigment, coloration of the medium is observed. In some cultures this coloration is black; in others brown. In those cultures which fail to produce pigment the solution becomes thick and milky in appearance. Pure cultures maintain an alkaline reaction in the absence of calcium carbonate in the medium throughout growth and emit a rather pleasant odor.

A rather interesting phenomenon was often noted in these liquid cultures when treated with a lead-acetate solution. If the heavy white membraneous precipitate which forms is collected on a filter, it gradually darkens and within several minutes is black. The reaction resembles an enzymatic process. No attempt was made to ascertain whether or not this process is connected with the characteristic pigment production in the living cells.

The yield, or amount of growth, was determined by centrifuging 50 cc. of the culture until the supernatant fluid was clear. This was decanted off, care being taken not to disturb the precipitate cells. The centrifuge tube containing this precipitated growth was placed in a drying oven and desiccated for eight hours at 75° to 80° C. to a constant weight. Duplicate determinations of each culture were made. The yield is recorded as a percentage of sugar originally present in the medium.

A daily comparison of the nitrogen assimilation, dextrose fermentation, and the yield of three cultures of Azotobacter is summarized in Table II. Four flasks containing 250 cc. of dextrose medium were used for each culture. All were aerated vigorously at a temperature of 30° C. One flask of each culture was removed daily for analysis. The medium contained 1 gm. of dextrose per 100 cc.

TABLE II.—*Yield rate of dextrose fermentation and nitrogen fixation by Azotobacter*

	1 day.				2 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19-399		10 B	19	19-399	
Nitrogen per 100 cc. (mgm.)	1.82	4.31	5.60	3.91	8.77	8.21	10.20	9.06
Dextrose per 100 cc. (gm)	.....	.49	.67	.....	.478	0	.06	.....
Yield per 50 cc. (gm.)	.0920	.0743	.0319	.....	.0901	.1939	.1281	.....
	.0750	.0717	.0154	.....	.0784	.1741	.1198	.....
Yield (per cent)	16.70	14.50	5.73	12.31	16.85	34.80	24.79	28.81

	3 days.				4 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19-399		10 B	19	19-399	
Nitrogen per 100 cc. (mgm.)	9.36	13.72	12.50	11.86	12.0	14.43	12.90	13.11
Dextrose per 100 cc. (gm.)	.05	0	0	.....	0	0	0	.....
Yield per 50 cc. (gm.)	.0841	.1487	.1234	.....	.1043	.1317	.1017	.....
	.....	.1595	.....	.....	.0889	.1528	.1149	.....
Yield (per cent)	16.82	30.82	24.68	24.10	19.32	28.45	21.66	23.14

There occurs a gradual increase in the quantity of nitrogen fixed. The average quantity fixed for the three cultures was for the first day 3.91 mgm., for the second day 9.06 mgm., for the third day 11.86 mgm., and for the fourth day 13.11 mgm., per 100 cc. of medium. The characteristic rapid disappearance of dextrose occurred. It was practically all consumed by the three cultures within two days.

The average yield of the three cultures for four consecutive days was as follows: 12.31 per cent, 28.81 per cent, 24.10 per cent, and 23.14 per cent of the dextrose originally present in the medium.

The effect of different quantities of dextrose upon nitrogen fixation and the yield was determined for media containing 0.5 per cent and 1.0 per cent dextrose. Flasks containing 250 cc. of medium were seeded with Azotobacter and aerated for four days at 30° C. Total nitrogen and yield determinations were made at the end of the incubation period. The results for the two cultures studied are recorded in Table III.

TABLE III.—*Effect of varying quantities of dextrose upon yield and nitrogen fixation by Azotobacter*

Culture No.	0.5 per cent dextrose.			1 per cent dextrose.		
	Weight.	Yield.	Nitrogen per 100 cc.	Weight.	Yield.	Nitrogen per 100 cc.
	Gm.	*Per cent.*	*Mgm.*	*Gm.*	*Per cent.*	*Mgm.*
19	0.0413 .0437	17.0	9.88	0.1014 .1153	21.66	16.38
232	.0322 .0357	13.58	7.57	.1143 .1282	24.24	16.65
Average	.....	15.29	8.72	.....	22.95	16.57

The largest yield for the quantity of dextrose used was obtained from the medium containing 1 per cent dextrose. The average yield for the two cultures was 22.95 per cent for this medium as compared with 15.29 per cent for the cultures cultivated in the 0.5 per cent dextrose medium. In the 1.0 per cent solution the cultures fixed on an average 16.57 mgm. of nitrogen per gram of sugar; and in the media containing 0.5 per cent dextrose, an average of 8.72 mgm. of nitrogen per half gram of dextrose. In other words, the rate of fixation was practically the same in both solutions.

A similar experiment was made with media containing approximately 0.5 per cent, 1.0 per cent, and 1.5 per cent of dextrose, respectively. The yield with each percentage of dextrose used, the quantity of nitrogen fixed, and the rate of dextrose fermentation were determined every two days for a period of six days. The cultures were vigorously aerated at 30° C. The dextrose content of the control media by analysis was 0.60 per cent, 1.1 per cent, and 1.5 per cent, respectively. The results are summarized in Table IV. The percentage of yield is based upon the quantity of dextrose originally present in the media.

TABLE IV.—*Comparison of various quantities of dextrose in culture No. 19 upon yield, nitrogen fixation, and dextrose fermentation.*

	0.6 per cent dextrose.			1.1 per cent dextrose.			1.5 per cent dextrose.		
	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Weight (gm.)	0.0354 .0328	0.0399	0.0692 .0669	0.0766 .0574	0.1506 .1280	0.1245 .1317	0.1190 .1354	0.2383 .2713	0.2036 .1928
Yield (per cent)	11.3	13.3	22.6	12.1	25.3	23.3	16.9	33.9	27.0
Dextrose (gm.)	.20	Trace	0	.68	Trace	0	.98	Trace	0
Nitrogen (mgm.)	6.37	9.75	9.70	7.41	17.29	18.72	9.1	17.68	26.0

The results show that the yield increases with an increase in the concentration of dextrose in the media. An average of the three periodical analyses for each medium gives a yield of 15.7 per cent for the 0.6 per cent dextrose solution, 20.2 per cent for the 1.1 per cent solution, and 25.9 per cent for the 1.5 per cent solution.

The total nitrogen content of each medium at the completion of the experiment reveals the fact that nitrogen fixation occurred at the rate of 16.1 mgm. of nitrogen per gram of dextrose in the 0.6 per cent dextrose solution, 17.0 mgm. of nitrogen per gram of dextrose in the 1.1 per cent dextrose solution and 17.3 mgm. of nitrogen per gram of dextrose in the 1.5 per cent dextrose medium.

The dextrose disappeared rapidly from all solutions, being entirely consumed by the sixth day.

UTILIZATION OF MOLASSES

The waste molasses from a sugar factory should offer an available source of energy for Azotobacter. Hence an experiment was conducted in which molasses was substituted for dextrose in the medium, and the azofication ability of cultures was determined in this medium. The medium contained 1 per cent molasses, or by analysis 0.51 per cent of invert sugar. The quantity of nitrogen fixed per 100 cc. of medium for five cultures aerated for six days was as follows:

	Mgm.
1 B	1.5
2 B	3.3
5 B	3.3
12 B	3.4
232	3.6

The results show an average net gain of 3.03 mgm. of nitrogen per gram of molasses. A gram of molasses represents only 0.51 per cent of invert sugar, giving, therefore, a rate of fixation of 6.06 mgm. of nitrogen per gram of invert sugar.

To determine the yield from molasses, 250 cc. of the 1 per cent molasses medium was inoculated with culture No. 19 and aerated for four days at 30° C. The medium was analyzed for sugar, total nitrogen, and yield. The results are given in Table V.

TABLE V.—*Yield, nitrogen fixed, and sugar fermented from molasses*

Sugar per 100 cc.		Nitrogen per 100 cc.			Weight per 50 cc.			Yield.
Control.	Culture.	Gross.	Control.	Net.	Gross.	Control.	Net.	
Gm.		*Mgm.*	*Mgm.*	*Mgm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*
0.50	Trace.	10.53	8.15	2.38	0.0799 0.0935	0.0063 0.0149	0.0761	30.44

The total nitrogen content of different samples of molasses has varied from about 6 to 12 mgm. per gram of molasses. This nitrogen appears to be in a soluble form, since lead acetate fails to precipitate it. On the other hand, if a lead acetate solution is added to an Azotobacter culture cultivated in this molasses medium, a heavy membraneous precipitate forms. An experiment, therefore, was arranged to ascertain whether

this nitrogen had been synthesized into a more complex nitrogenous substance by the growth of Azotobacter.

Eight flasks containing 250 cc. of molasses medium were inoculated, four with culture No. 19 and four with culture No. 232, and aerated thoroughly for eight days.

The contents of the four flasks of each culture were mixed, and total nitrogen determinations were made. The remaining portion was precipitated with a lead-acetate solution and filtered. This filtrate was perfectly clear. Total nitrogen determinations were likewise made on this filtrate. The control medium was treated in a similar manner.

The average results for all duplicate determinations are as follows:

Control	Medium 6.71 mgm. nitrogen per 100 cc. Filtrate, 6.40 mgm. nitrogen per 100 cc.
Azotobacter No. 19	Culture 9.52 mgm. nitrogen per 100 cc. Filtrate 3.8 mgm. nitrogen per 100 cc.
Azotobacter No. 232	Culture 10.30 mgm. nitrogen per 100 cc. Filtrate 0 mgm. nitrogen per 100 cc.

From these results it is evident that Azotobacter is capable of synthesizing the nitrogen present in the molasses into more complex substances as well as assimilating nitrogen from the air.

UTILIZATION OF STRAW

Pringsheim and Lichtenstein (*13*) report an investigation the purpose of which was to enrich straw concentrate with protein by means of fungi. Hydrolyzed straw was spread out in thin layers and inoculated with an Aspergillus. The protein content of the straw increased from 0.9 per cent to 8.0 per cent within a week Digestion experiments with the food proved satisfactory.

The utilization of vegetable tissues as available sources of energy for Azotobacter has been reported by Murray (*11*) and Hutchinson (*12*).

Experiments were undertaken in the present case with a view to increasing the protein content of wheat straw by Azotobacter. To each 200 cc. of a dextrose, and also a molasses medium, there was added 1 per cent of a good grade of wheat straw which had been finely ground in a mill. This was seeded with Azotobacter cultures and aerated for varying lengths of time. Total nitrogen determinations were made in duplicate on the entire contents of the culture flasks. To note the effect of straw in the medium, similar determinations were made on cultures containing no straw.

The results are recorded in Table VI for three cultures aerated in the dextrose solution and in the straw dextrose medium for four days.

TABLE VI.—*Effect of straw on azofication.*

Culture No.	Dextrose-Ashby medium (200 cc.).			Dextrose-Ashby and straw medium (200 cc.).		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1 B	1.4	7.80	6.4	9.4	20.01	106.1
10 B	1.4	8.08	6.68	9.4	19.86	10.46
232	1.4	9.97	8.57	9.4	22.36	12.96

The net gain of nitrogen fixed by all the cultures in the straw solution exceeded the net gain in the dextrose by an average of 4.13 mgm.

The experiment was repeated, and a daily analysis of the cultures was made. The cultures in this case were aerated for four days. The data are tabulated in Table VII.

TABLE VII.—*Effect of straw on azofication*

Culture No.	Medium.	Nitrogen in control.	1 day.		2 days.		3 days.		4 days.	
			Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
10 B.	D. A. 200 cc.[1]	1.0	4.55	3.55	18.46	17.46	19.20	18.20	24.96	23.96
10 B.	D. A. S. 200 cc.[2]	10.07	10.40	.33	20.93	9.87	30.29	20.22	29.44	19.37
19...	D. A. 200 cc.[1]	1.0	8.38	7.38	15.14	14.14	24.70	23.70	25.02	24.02
19...	D. A. S. 200 cc.[2]	10.07	13.78	3.71	25.02	14.95	39.32	29.25	42.83	32.76

[1] Dextrose-Ashby 200 cc. [2] Dextrose-Ashby and straw 200 cc.

In general, the results are similar to those reported in the previous experiment. However, culture 10 B showed an approximate net gain of 2.0 mgm. at the end of the third day in the straw culture and a loss of over 4.0 mgm. of nitrogen on the fourth day. An average of the two cultures indicates a net gain of 4.15 mgm. for the straw medium for the entire period of the test.

As expected, a greater net gain of nitrogen was recorded for the dextrose solution than for the straw medium for the first two days. This indicates that the straw is not utilized until all of the greater part of the dextrose is consumed.

Calculating all the nitrogen in the wheat straw as protein nitrogen indicates a protein content of 3.14 per cent. In a similar manner, calculating the nitrogen in the straw solution as protein nitrogen indicates an average protein content of the straw from the two cultures of 11.29 per cent, thus giving a net gain of 8.15 per cent protein.

However, but little of this net gain of protein can be attributed to the influence of the straw, for an average net gain of only 2.07 mgm. of nitrogen per gram of straw was noted in the straw dextrose media.

A summary of both experiments indicates an average net gain of only 1.54 mgm. of nitrogen per gram of straw when added to dextrose medium.

In another experiment three cultures were aerated for four days in a molasses solution and three other cultures in a straw-molasses solution. The results are recorded in Table VIII.

TABLE VIII.—*Effect of straw and molasses on azofication*

Culture No.	Molasses medium (200 cc.).			Molasses and straw medium (200 cc.).		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1 B.....	17.54	19.69	2.15	25.37	28.98	3.61
10 B.....	17.54	21.50	3.96	25.37	33.88	8.51
232.....	17.54	18.57	1.03	25.37	28.55	3.18

The data show a net gain of nitrogen in favor of the straw medium for all cultures. The average net gain of nitrogen for all cultures grown in the molasses solution was 2.38 mgm. and for those grown in the straw solution 5.10 mgm. This gives an average of net balance in favor of the straw media of 2.72 mgm.

Another experiment was conducted in which two flasks containing 200 cc. of straw-molasses solution were seeded with two Azotobacter cultures and aerated for three days. Ferric-sulphate solution was added to precipitate the protein in each culture flask. The entire contents of each flask were then placed upon a filter, and the residue was collected. This precipitate was desiccated and total nitrogen determinations were made. A control medium was treated in a similar manner. The results are presented in Table IX.

TABLE IX.—*Effect of straw and molasses on azofication*

Culture No.	Total nitrogen per gram of straw.	Net nitrogen per gram of straw.
	Mgm.	*Mgm.*
Control	11.18	.....
232	15.41	4.23
1 B	15.84	4.66

The experiment shows an average net gain of 4.44 mgm. of nitrogen per gram of straw, or 2.77 per cent protein.

SUMMARY

(1) The protein content of Azotobacter growth obtained from a solid medium was found to be 11.81 per cent, while that collected from a liquid culture was 30.56 per cent.

(2) The yield of cells increased with the quantity of dextrose in the medium. An average yield of 15.7 per cent, 20.2 per cent, and 25.9 per cent of the sugar was obtained from medium containing 0.6 per cent, 1.1 per cent, and 1.5 per cent dextrose, respectively.

(3) The relative quantities of nitrogen fixed per gram of dextrose for the three different percentages of sugar were similar—namely, 16.1 mgm., 17.0 mgm., and 17.3 mgm., respectively.

(4) When molasses was used as a source of energy for Azotobacter development there was obtained a yield of cells equal to 30.44 per cent of the sugar in the molasses. This gives a higher percentage yield for the molasses sugar than for dextrose. If the moisture content of the molasses is considered, the percentage yield from the actual molasses exceeds the yield from dextrose.

(5) Azotobacter is able to convert the soluble nitrogenous substances present in molasses into more complex protein, as well as to utilize the molasses as a source of energy for the fixation of atmospheric nitrogen.

(6) The addition of straw to the dextrose or molasses medium did not cause any appreciable increase in the quantity of nitrogen fixed.

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